

STAPEDECTOMY

A study on experimental grafting at the  
oval window

A Ch.M. Thesis submitted to  
the University of Edinburgh

by

B.H. Colman, M.B., Ch.B., F.R.C.S.E.

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## I. HISTORY

The concept of mobilising or removing the stapes in order to improve the transmission of sound to the oval window is by no means new though the limitation of such procedures to the treatment of otosclerosis and their incorporation with various methods of ossicular reconstruction represent recent refinements.

Stapes fixation as a cause of deafness was first reported post-mortem in 1735 by Valsalva (1) in the course of a detailed account of the anatomy of the ear (see Appendix I, page 110). Interest was rekindled by Toynbee (2) in 1841 who in the course of dissecting a series of 41 ears of persons known to have been deaf, discovered a case of bilateral stapes ankylosis. He found the ankylosis "to be produced by an expansion of the base of the stapes" which formed an "oval protuberance which was smooth, and of an opaque white, and firmly adherent to the vestibular parietes." He regarded the condition as "a disease of the stapes, the walls of the vestibule being perfectly healthy." During the subsequent years he continued his investigations and by 1874 (3) had collected 189 cases of stapes ankylosis or rigidity in the course of 1149 autopsies. His classification into six degrees depending on the site and extent of the lesion is still suitable for clinical use.

Surgical interest in the ossicles and oval window region preceded /

preceded by some years an exact appreciation of the pathology of deafness. The earliest surgical ventures for the relief of deafness due to ossicular fixation are probably those of Kessel (4, 5) reported in 1876 and 1877 and based on cases seen in the previous five years. He was aware of Toynbee's work and had himself examined 1000 ears at autopsy. Though dealing frequently with the effects of healed suppuration he appreciated that stapes ankylosis was a separate entity and that two functioning labyrinthine windows were necessary for good hearing. His earlier operations of removal of drum, malleus and incus led him eventually to an attack on the stapes itself. In the latter operation, after separating the incudo-stapedial joint he freed the stapes by using a fine knife around its edges until perilymph appeared. He felt however that extraction of the stapes was better so that the fixed ossicle was replaced by the formation of a membrane across the oval window. He was not aware of anyone else doing similar work and at this distance in time one cannot but admire his boldness and skill particularly in the absence of those facilities which to-day are considered essential for working in this area.

His pioneering work was followed by a report from Boucheron (6) in 1888 who described 60 cases. After separating the incudo-stapedial joint he mobilised the stapes by gentle traction, pressure and rocking movements. Favourable results were obtained especially in the earlier stages of fixation.

A further series of papers by Miot (7) two years later dealt /



dealt with 200 cases of tympanotomy for various types of middle ear deafness and as a text on stapes mobilisation is still remarkably fresh. He describes lucidly the anatomy and its variations, the indications and contra-indications for surgery, compares the techniques of Kessel, Schwarze and Gelle with his own and describes the various accidents and complications (including injury to the chorda tympani, fracture of the crura and subluxation of the stapes footplate).

He appreciated the importance of retaining the incudo-stapedial joint and the crura intact and was able to report improvement in 45% of what were probably otosclerotic patients. Infection occurred only four times and was confined to the middle ear. Like Boucheron he concluded that the most suitable cases were those with early fixation and the worst those with extensive ankylosis or cochlear degeneration. He expressed the view that the operation was the best available for the treatment of stapes fixation but as now it was appreciated that whilst mobilisation could produce a permanent hearing improvement it very often did not and the results were rather unpredictable.

During the next few years the most important papers were mainly from American authors. Jack (8) in 1892 reported on 16 cases most of which obtained improved hearing. Though some of the cases were undoubtedly otosclerosis many appear to have been for the results of suppuration, occasionally still active. The operations varied, sometimes including removal of drum, malleus and incus, sometimes being confined to the stapes and clearly /

clearly including complete removal of its footplate in several cases. While not attempting to explain the results, his impression was that improvement simply resulted from removal of the mechanical obstruction to the sound waves. He remarked that the results were strangely at variance with repeated statements that footplate removal destroyed hearing and regarded the procedure as offering "the most encouraging outlook for the permanent relief of deafness than any operation heretofore done." He published further favourable results later in the same year, still without any complications apart from a short period of vertigo (9).

Blake (10, 11) in papers also published the same year was more critical and logical in his approach than his contemporaries appear to have been. Myringotomy and myringectomy (Kessel's earliest operations) he dismissed as being based on inadequate knowledge. Removal of the malleus and incus he regarded as being unscientific and unnecessarily violent and suggested that any improvement resulted from accidental mobilisation of the stapes, the ossicle primarily at fault in most cases of non-suppurative deafness. He felt that complete removal of the stapes was superior to mobilisation in its results but reported frequent difficulty from crural fracture.

The following year, 1893, saw further encouraging reports by Burnett (12) though in his cases footplate removal was probably not achieved and also again by Jack (13) whose aim now was always to remove the footplate if possible. He claimed good /

good results in cases diagnosed as healed otitis media suppurativa, otitis catarrhalis adhaesiva and otitis media insidiosa (sclerosis), though footplate extraction in the latter condition was often impossible due to apparent bony fixation and the results were correspondingly poorer.

Further papers by Dench (14), Kessel (15) and Jack (16), published in 1894 and 1895, again reported promising results from mobilisation and stapedectomy. The last mentioned paper which Jack read to the Boston Society for Medical Observation in October 1894 was based on 60 cases, some with a three year follow-up. He again emphasised the difficulty of extracting the footplate in "sclerosis" and for the first time gave warning of labyrinthine degeneration (which he thought the result of haemorrhage) being caused by stapedectomy.

At approximately this time two factors undoubtedly had a dampening effect on the enthusiasm of otologists interested in this type of work.

Firstly, improved understanding of the various diseases and their pathologies was developing. Politzer (17) described the pathology and histology of stapes ankylosis; he emphasised that Toynbee's cases and his own represented a primary disease of the labyrinthine capsule, not of the stapes. His work was based on a study of 16 cases varying from a small anterior focus to obliteration of the oval window. Discussing stapedectomy, he pointed out the impossibility of achieving footplate removal if the disease was advanced, since the crura would invariably fracture first. He also drew attention to the /

the futility of hoping for long term hearing improvement from simple mobilisation because of the inevitable bony re-union. He felt that "in spite of the great advances being made in otology in recent years, the disease will not be controlled till the causative bone disease can be controlled." And he doubted if it ever would be. In this respect the answer to otosclerosis is little nearer even to-day and treatment remains essentially palliative.

Secondly, in spite of the many encouraging reports, a number of otologists had experienced alarming results. Blake at the 1894 International Congress in Rome, quoted by Politzer and by Leland (18) had personally reported on 22 patients who had been made extremely deaf or stone deaf in the operated ear after stapedectomy. Blake's experience of disaster cannot have been unique and one cannot but wonder about the eventual fate of those persons whose stapes were completely removed in the presence of a chronic otorrhoea.

Soon after this date these operations appear to have been completely abandoned and reports concerning stapes surgery disappeared apart from a series of papers by Feraci (19, 20) a few years later when satisfactory hearing improvement was again claimed. Siebenmann (21) finally brought this era to an end by his condemnation of stapes surgery in his address to the International Congress in 1900, the effects of which were felt for the next half century. Even as late as 1937 Sourdille (22) stated: "The footplate of the stapes is so fixed by an osseous process and the crura so wedged in by the borders of the /

the deformed oval window that extraction of the ossicle is practically impossible. If one exerts traction on its crura they are certain to fracture and there is no other way of raising the footplate. Besides, even if this could be done, the large communication established between the tympanum and labyrinth will permit infection to enter and thus lead to total deafness. It was this simple conception which led in 1900 to an official condemnation of the operation which was so severe that we are still influenced by it."

Surgical endeavour meantime was being concentrated on fenestration of the posterior labyrinth and culminated in Lempert's (23) one stage procedure of 1938, which with modifications became the cornerstone of surgery for otosclerosis. It is interesting to note that though Lempert was primarily responsible for perfecting the fenestration operation, by a strange turn of fate it was also his originality that brought about a return of interest in the oval window. In 1946 he demonstrated a permeal approach to the middle ear utilising a posterior tympano-meatal flap, for use in carrying out a tympanosympathectomy (24), and later he used the same route for extraction of the stapes for decompression of the labyrinth in Meniere's disease (25).

The success of fenestration in showing that the labyrinth could safely be opened, together with the advent of chemotherapy, improved local anaesthesia, lighting and magnification did much to facilitate further changes in the treatment of otosclerosis.

Lempert's /

Lempert's permeal approach to the stapes was followed exactly by Rosen (26) when palpating the stapes to determine its degree of fixation prior to a proposed fenestration of the lateral semicircular canal. It was this accidental rebirth of stapes mobilisation on 3rd April 1952, in the absence of any knowledge of the early pioneering, that led to the present renaissance in oval window surgery which continues to stimulate so much interest, so many ingenious variations and not a little controversy.

## II. DISCUSSION OF THE CURRENT PROBLEM

That oval window surgery continues to stimulate so much interest arises in part from certain intrinsic disadvantages of classical fenestration of the lateral semicircular canal. These disadvantages persist in spite of recent innovations and over a period of 25 years fenestration has reached the point where it perhaps cannot undergo further radical improvement. In contrast, oval window surgery is still in its infancy and it is not unreasonable to assume that methods will be evolved to combat its present hazards as well as any causes of long term failures which may become apparent. The acoustic advantages of utilising the transformer mechanism of the middle ear are so great that efforts will continue to re-establish sound conduction to the oval window as the treatment of choice.

Although an improvement in hearing is virtually certain and in fact is predictable within close limits after canal fenestration a degree of conductive loss is almost inevitable. Those cases in which the conductive loss is completely (or almost completely) eradicated are very few in number and difficult to explain on the basis of current ideas of hearing physiology. Davis and Walsh (27) estimated the usual deficit to be at least 20 decibels whilst Shambaugh (28) in his cases found it averaged 25 decibels for the speech frequencies. The recent work by Lawrence (29) on middle ear physiology confirms the /

the 25 decibels advantage provided by the normal middle ear anatomy.

This residual conductive loss imposes certain limitations on the selection of cases for canal fenestration as well as on the actual results obtained. It also means that a certain proportion of patients whose cochlear reserve is potentially adequate still require amplification. With complete, or almost complete, removal of the conductive element this proportion can be correspondingly diminished though even with an ideal result there will remain a percentage whose poor cochlear function still keeps them below the Social Adequacy Level. Briefly, candidates for fenestration should have an air-bone gap of at least 25 db. for the speech frequencies (or 35 db. after correction for the Carhart phenomenon) and a cochlear reserve adequate to ensure a reasonable result i.e. bone conduction at 20 db. or better for 500 and 1,000 c.p.s. and 30 db. or better for 2,000 c.p.s. Fenestration is seldom advisable for unilateral otosclerosis with normal hearing on the other side as adequate binaural hearing can seldom be attained. These criteria have recently been stressed again by Shambaugh (30) and others.

Furthermore, the presence of a cavity calls for regular attention once or twice yearly to keep it healthy. A small minority of cavities, usually from neglect, become infected maybe months or years after operation and can prove obstinate to heal, especially if an area of osteitis develops. A cavity imposes certain limitations on the activities of the patient, particularly /



particularly in the realm of sport and recreation, which can be a severe handicap in the young and active especially in warmer climates. Recent innovations in which a pedicle flap of muscle and sheath are employed to obliterate the cavity in tympanoplasty may be applicable to this problem but have yet to prove their value. The recent announcement by Rambo (31) of a new method of closed fenestration of the lateral semicircular canal, in which the fenestra is covered by a flap of the temporalis muscle brought into contact with the head of malleus is similarly of interest. In spite of these changes, canal fenestration will remain a major undertaking requiring a considerable stay in hospital, a considerable time to obtain complete healing and a considerable amount of rehabilitation.

Soon after its re-introduction, stapes mobilisation proved attractive in avoiding certain of these drawbacks and it was found that in spite of the mediocrity of the results in the majority, the operation occasionally gave excellent long term results with complete air-bone closure as House and Glorig (32) pointed out. The potentialities of the method having been so demonstrated, subsequent rapid developments of the operation were designed to secure this result more often.

The resultant modifications in the last eight years have advanced along fairly logical lines from various mobilisation techniques involving pressure around the long process of incus and the head and neck of stapes to a variety of footplate procedures such as pressure, needling, chiselling and trephining.

As /

As in the last century, mobilisation finally led to partial and then total stapedectomy. The footplate may be replaced by a graft, the tissues employed having been skin (33) and later vein (34) in Shea's hands, or alternately connective tissue and adipose tissue as described by Schuknecht et al (35).

Alternately the oval window can be covered by a film of gel sponge to promote the development of a firm layer of blood clot over the exposed perilymph after the method of House (36).

The stapes suprastructure may be retained as in the method of Portmann (37) who objects to the introduction of foreign materials but if removed can be replaced by a prosthesis of polythene as described by Shea (33, 34) or by wire of stainless steel or tantalum, after the method developed by Schuknecht (35).

#### RELATED EXPERIMENTAL WORK

The amount of animal experimentation has been meagre, partly perhaps due to the difficulties involved. A considerable amount of new information was presented at the Detroit International Symposium on otosclerosis in November 1960 and is due for publication in July 1961, though even there much of the new work reported did not involve stapedectomy and grafting except indirectly.

The effect of surgical trauma in the oval window area has been examined by Bellucci and Wolff (38, 39) in some of whose cats and monkeys the stapes was removed entirely. They implanted /

implanted no graft but found nevertheless that a membrane sometimes formed across the window and can partly prevent degeneration of the organ of Corti. They also noted that if instrumentation in the window was excessive, new bone formation sometimes occurred. This clearly has an application in human stapes mobilisation. Singleton and Schuknecht (40) in a similar experiment on ossicular trauma also describe the methods of ossicular healing and report in more detail on the internal ear results.

The first paper on the behaviour of grafts in this area was that of Myers et al. (41) who reported on the behaviour of vein when placed across the oval window after stapedectomy in cats. Unfortunately, no endeavour was made to section the intact temporal bones so that the information gained was of limited value and gave no indication of the state of the middle or internal ear. They found that the grafts had greatly thickened, mainly due to invasion by collagenous tissue.

A recent paper, again from Bellucci and Wolff (42) reports their findings in three types of experiment:

- (a) stapedectomy with insertion of a graft and polythene prosthesis;
- (b) insertion of gel foam across the window after stapedectomy (partial or complete) or after footplate fragmentation;
- (c) complete removal and then replacement of the stapes.

The results in the first type of experiment were uniformly poor, /

poor, an aseptic necrosis of the graft occurred in all six animals and fibrosis was severe.

The main conclusions they draw from the series of experiments are (i) polythene (nine ears) was well tolerated but that the vein graft itself was responsible for fibrosis occurring; (ii) gel foam (eight ears) was better tolerated but still caused much fibrosis, there was a cochlear injury, possibly toxic; (iii) lesser degrees of footplate removal were associated with lesser degrees of cochlear injury.

This experiment represents the only one of importance available in the literature though they presented similar results at the Detroit Symposium.

The only report available on the appearance of other tissues which might be grafted into the oval window apart from vein is that of Colman (43) who briefly communicated certain of his findings to the Royal Society of Medicine in February 1960. The findings form a part of this thesis.

In its human application, the response towards an oval graft is closely bound with the tolerance of foreign materials used in reconstructing the ossicular mechanism. Information on this problem has been more forthcoming.

Withers (44, 45) found that polythene was well tolerated in the cat and Myers et al. (40) confirmed it as part of their experiment. They found that polythene quickly became enclosed in an envelope of mucosa and thus became "tied in" as an integral part of the middle ear mechanism. Hall, however, has /

has since observed in the re-opened human ear that a polythene tube becomes only partly enclosed by a mucosal sheath and is mainly fixed in position by a column of fibrous tissue filling its lumen between graft and lentiform process (46).

The tolerance of the ear to stainless steel and to tantalum wire has been examined by Schuknecht and Oleksiuk (47) working with cats. They found these materials to be entirely inert and to have become completely enclosed in a delicate mucosal sheath along their entire length in the middle ear. The complete acceptance of tantalum wire was particularly emphasised in one animal in which the wire had been inserted through the footplate: the only response by the labyrinth was to cover it with a layer of endothelium.

Wullstein (48) in contrast has expressed the view that foreign bodies of any type are usually rejected by the ear. Zollner is opposed to their use equally in tympanoplasty and in stapedectomy (49). He emphasises that though the foreign body may become enclosed in fibrous tissue or mucosa it can never become organically incorporated and for this reason he creates a new columella of bone when in the course of stapedectomy he finds that the stapes suprastructure cannot be utilised (50).

In spite of research carried out in recent years, it is apparent that much remains to be learned concerning the histopathology of the oval window region in relation to stapedectomy. Even so, stapedectomy with insertion of a prosthesis and graft has been described by Shea (34) as the "ultimate operation" for otosclerosis. /

otosclerosis. After a recent trial of transmeatal lateral canal fenestration with incudopexy, Ruedi has also expressed the view that stapedectomy offers the best chance of relieving otosclerotic deafness (51).

On the other hand, the operation has been severely criticised by otologists of long experience whose opinions must be respected. Walsh (52), in discussing the merits of fenestration and stapes surgery, has justifiably deplored the persistent lack of experimental work and drawn attention to the dangers of changes in technique involving the insertion of foreign materials and pulverisation or removal of the footplate without more animal research. He has undoubtedly expressed the anxieties felt by many otologists, yet comparatively little has so far been done to amend this state of affairs.

### III. THE CURRENT EXPERIMENT

The encouraging results in human work and the increasing clinical interest in the oval window suggested that an experimental enquiry would not be inopportune.

Though it is not possible to study otosclerosis in animals it is possible to devise animal experiments to study the effect of operations which are applicable to the disease. The result of stapes surgery depend also on the response of the middle and internal ear as well as the behaviour of the oval window and any material transplanted into it. Such experiments can provide information about the method of healing in a successful operation as well as an indication of the pathology resulting from failure, information which in human aural surgery can rarely be obtained directly.

An experiment was accordingly designed (i) to examine the results of a satisfactory stapedectomy and oval window graft and make a comparison of the appearances, behaviour and relative suitability of each type of graft; (ii) to examine the responses invoked in the middle and internal ear after a successful implantation; (iii) to examine the results of failure and its effects on the middle and internal ear. It was also hoped to gain experience in the course of these procedures which might be of help in human work of a similar nature and also to correlate the pathology of any failures to the human failures which have been reported.

In /

In choosing a suitable graft certain criteria clearly needed consideration: the tissue must be easily obtainable; it must be thin and pliable and should remain so; it must be incapable of producing any adverse effect in the middle ear and must be adaptable to the physiological conditions present in the middle ear (especially contact with air); it must be non-injurious to the labyrinth; it must be capable of preventing extension of a middle ear infection to the labyrinth. And if it is to be used in human stapedectomy operations it must be strong enough to support a prosthesis or capable of being incorporated in one.

With these requirements in mind and the realisation that others might become apparent later, six tissues were selected for investigation viz. skin, mucosa, vein, conjunctiva, connective tissue and adipose tissue.

Healthy adult cats, previously examined to exclude the presence of ear disease were used in the experiment and three operations were done with each type of tissue.

#### Method

Intra-peritoneal Sodium Nembutal, 1.5 ccs. of the veterinary preparation (containing 60 mg./cc.) was usually found to give satisfactory anaesthesia. Occasionally an extra 0.25 cc. was needed to abolish the response to painful stimuli, inhalation anaesthesia was not used. A temporary tracheotomy was fashioned routinely to ensure an adequate airway and removal of secretions during the course of the operation.

The skin was shaved around the side of the head and neck and /



and the animal positioned in a specially designed head holder. After thorough skin preparation the animal was towelled up and the operation carried out with the strictest attention to sterility. A supra-auricular incision was employed, temporalis identified and retracted to expose the cartilaginous meatus. Using a Zeiss operating microscope and an electrically driven drill, a burr-hole was made into the temporal bone immediately above the bony meatus.

The opening into the attic was then enlarged anteriorly to expose the incudo-malleolar joint and part of the malleolar head. Posteriorly, as much bone was removed as necessary to ensure an adequate view of the oval window recess after removal of the incus. The amount of bone removal was limited (a) by the presence of the facial nerve which in the cat opens on to the lateral surface of the skull posterior or postero-inferior to the bony auditory canal, (b) by the necessity of avoiding so much bone removal that the soft tissues prolapsed into the attic during healing.

With the head in an oblique position adequate access was obtainable often with very little enlargement of the initial atticotomy. Constant care was necessary during this part of the operation to avoid damage to the bony annulus and to the drum. Under high magnification and using specially modified instruments the stapedius tendon was divided and the mucosa pushed back from the footplate. After loosening the annular ligament with special needles the stapes was sometimes sufficiently loosened to be extracted intact. In the event of crural /

crural fracture the footplate was penetrated with a needle and the footplate removed in fragments by fine hooks. As far as could be certain at the time of operation complete removal was obtained on each occasion but in fact was not always so. The utmost gentleness was exercised throughout. An autogenous graft, meantime having been tailored to suitable dimensions, was inserted and spread across the window. The oval window in the cat measures approximately 1.3 mm. in its long diameter, the graft was cut with just sufficient overlap to prevent it falling through the window into the labyrinth yet small enough to allow an accurate fit deep in the window recess. A single piece of gel foam was employed to give support. On occasions the amount of perilymph overflow was troublesome and caused the immediate washing-out of the graft; there was no alternative but to wait till the flow ceased in these animals.

The incision was closed in layers and the tracheotomy discontinued before returning the animal to its cage. Procaine penicillin was given intramuscularly for a week in a dosage of 100,000 units once daily.

After usual survival periods of 16-18 weeks the animals were sacrificed by perfusion with Ringer's solution followed by Heidenhain-Susa solution as a fixative.

The temporal bones were removed and prepared for histological examination as follows:

1. Fixation: Heidenhain-Susa solution 48 hours.
2. Decalcification: 5% trichlor acetic acid (degassed)  
14 days
3. Neutralisation: 5% sodium sulphate (degassed)
4. /

4. Dehydration: 35% ethyl alcohol (degassed) 1 day  
                   50% " " " " "  
                   60% " " " " "  
                   70% iodised " " " "  
                   80% " " " " "  
                   95% " " 3 days (changed daily)  
                   100% " " " " " "
  
5. Impregnation: alcohol and ether (equal parts) 3 days  
                   (changed daily)  
                   then 1.5% celloidin (dissolved in alcohol-  
   ether) 3 days  
                                   3% celloidin (dissolved in alcohol-  
   ether) 2 weeks  
                                   6% celloidin (dissolved in alcohol-  
   ether) 2 weeks  
                                   12% celloidin (dissolved in alcohol-  
   ether) 3 weeks

After embedding the bones and hardening by air exposure, the blocks were cut in the horizontal plain at 10 microns and stained with haematoxylin and eosin.

The animals are identified as follows in the description of results and in the discussion:

Skin grafted ears: Sk.1. Sk.2. Sk.3.

Mucous membrane grafted ears: Mn.1. Mn.2. Mn.3.

Vein grafted ears: Vn.1. Vn.2. Vn.3.

Conjunctiva grafted ears: Conj.1. Conj.2. Conj.3.

Connective tissue grafted ears: CT.1. CT.2. CT.3.

Adipose tissue grafted ears: Ft.1. Ft.2. Ft.3.

The numerals 1 to 3 do not necessarily indicate the order in which animals of each group were grafted.

#### IV. DESCRIPTION OF RESULTS

Two deaths occurred in the course of the experiment, both from causes unconnected with grafting. These procedures were therefore repeated to bring the total back to three grafts of each type.

##### I. CLINICAL RESULTS

Generally the animals were little upset by the operation. In no case did signs of an acute labyrinthine destruction occur and signs of vestibular disturbance were absent or very slight. Within four hours of the end of the operation animals were frequently able to stand but usually loathe to walk and in any case were presumably still under the influence of Nembutal. Post-operatively, first degree nystagmus was observed on the day of operation in approximately a third of the animals which could be examined. In only two animals did it persist till next day.

Disturbances of posture, particularly head or tail deviation, which in the cat are evidence of permanent vestibular damage, were not observed as a result of the operation. Jumping from a height was performed with agility equal to that of unoperated caged animals.

One animal, though entirely undisturbed after the satisfactory insertion of a mucosal graft, became suddenly and severely ataxic and showed all the signs of acute vestibular failure /

failure on the 44th day. This animal was sacrificed soon afterwards. The findings are described in the next section and illustrated in figure 8 (animal Mn.1).

No exact tests of hearing were made in any animal and in any case the sound-conducting mechanism of the middle ear was interrupted by the operation. It can, however, be said that the animals were still capable of responding to voices and "meaningful sounds" of reasonable intensity with the operated ear.

## II. HISTOLOGICAL RESULTS

Because of the number of observations the histological appearances can most conveniently be presented by first recording the appearances of the actual grafts, then examining the associated changes in the middle and internal ear and finally correlating these findings as they appeared in each animal in turn. The normal anatomy is illustrated in figure 1.

### The Grafts

#### (a) Skin

Skin was obtained from the deepest part of the concha or from the external auditory meatus, these areas being relatively hairless. A thin razor graft was employed. In all three ears the behaviour of the graft was essentially similar; the results are as follows:

Animal Sk.1 /

Animal Sk.1

The graft has proliferated to form an epidermoid cyst (fig. 2). That part of the cyst exposed to the middle ear cavity is covered by a delicate layer of collagen and a layer of normal middle ear mucosa. The cyst appears to have been formed as a result of the inclusion of a small amount of the basal cell layer of the epidermis at the edge of the graft, a small amount of underlying dermis could be identified at the posterior edge of the window. The basal cell layer has apparently proliferated, burrowing into the submucous connective tissue layer until the growing edges have finally been thrown back upon themselves and united in the confined space available.

In this animal the remarkable proliferative energy of the layer also has enabled the epithelium to invaginate itself around the superior edge of the oval window and form a small loculation within the labyrinthine vestibule (fig. 3).

All the normal epidermal layers were readily identifiable in the cyst wall except in that part bridging across the oval window itself. Here the graft has remained thin, and possibly is thinner than when inserted (fig. 4). There is no trace of the basal cell layer. The granulosal and prickle cell layers are so closely packed together that their differentiation is difficult. The corneal layer is readily identified. This part of the graft is lined on its vestibular surface by a delicate endothelial membrane consisting /

consisting of a single layer of thin, flattened cells. This layer was continuous with the endosteum of the labyrinth and doubtless derived from it.

#### Animal Sk.2

A cyst of exactly similar characteristics was found. There was again evidence that the graft had included a small amount of dermis and hence of the basal cell layer of the epidermis. The dermis was cut at the inferior part of the spherical cyst in transverse section and is illustrated in figure 5. It includes both sebaceous and sudoriferous glands as well as hair follicles, some of which still contained the hair cut across.

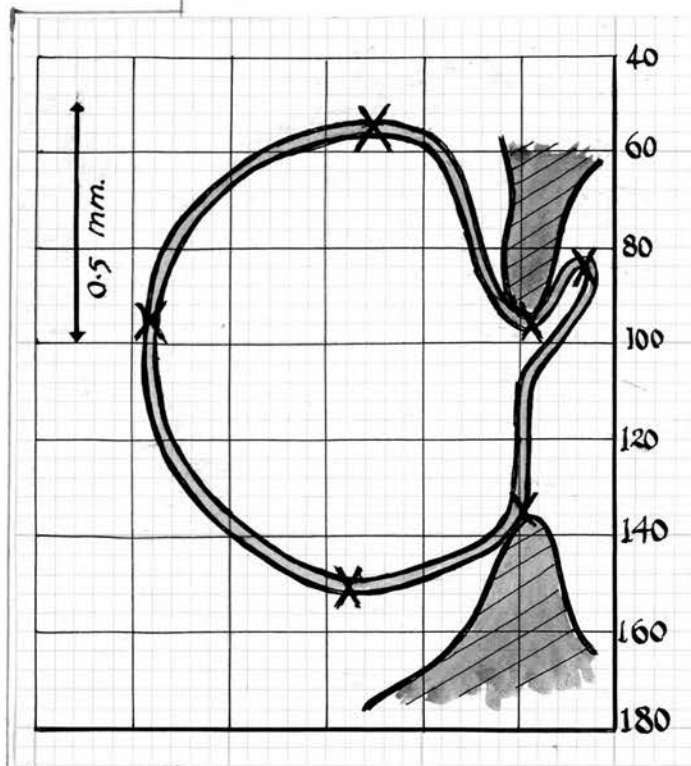
The cyst in this animal had not extended into the vestibule. As before it was covered by normal mucosa separating it from healthy middle ear. Posteriorly the graft had herniated outwards slightly, resulting in exposure of middle ear mucosa to the perilymph.

#### Animal Sk.3

A cyst was again present. There was no extension into the vestibule but the cyst has ruptured into the external auditory meatus forming a high postero-marginal and posterior attic perforation (fig. 6). Its epithelium has gained complete continuity with the skin of the meatus. As before, the cyst had no connection with the middle ear space and was covered by normal middle ear mucosa.

In addition to proliferating in area, the basal cell layer /

# DIAGRAM I

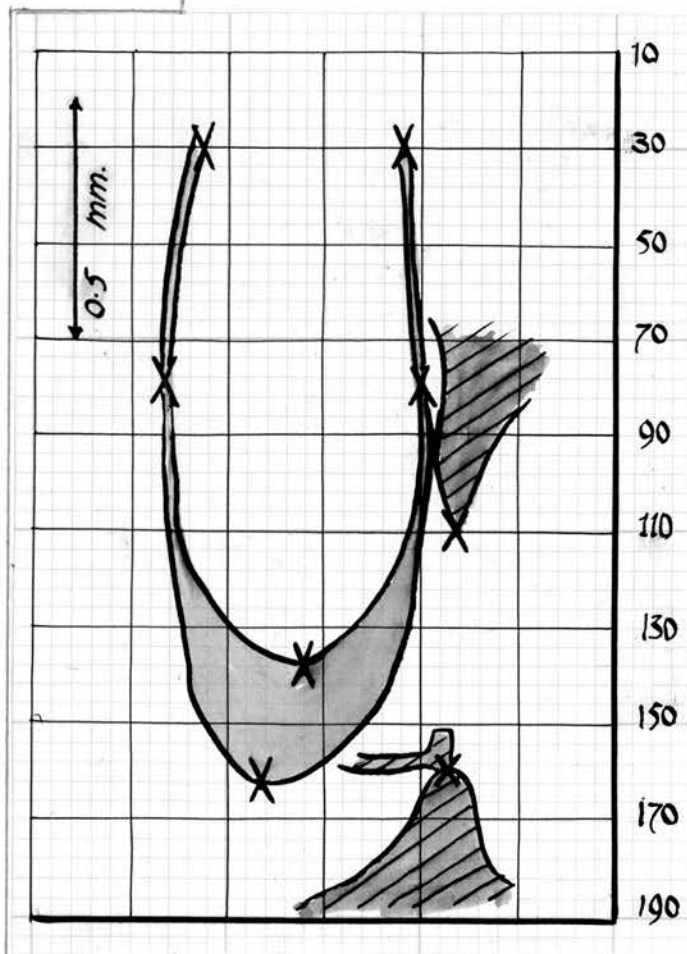


## Animal Sk.1

Vertical height of oval window 400 microns.

Vertical height of epidermoid cyst 950 microns.

Labyrinthine extension present.

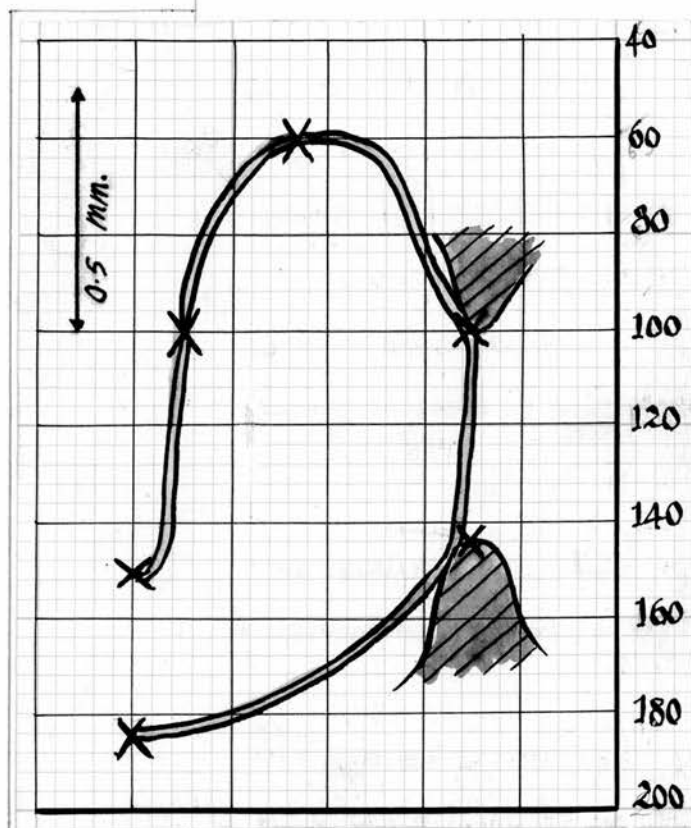


## Animal Sk.2

Vertical height of oval window 450 microns.

Vertical height of cyst 1050 microns at least.  
(sections superior to section 30 not available)





Animal Sk.3

Vertical height of oval window 420 microns.

Vertical height of cyst 1200 microns.

Attic and postero-marginal perforation.

The numbers beside each diagram indicate the serial number of the sections. It is presumed that the window was exactly in the vertical plane though in fact it was unlikely and the height is thus slightly fore-shortened. Thickness of each micro-section 10 microns. Diagram scale 1 inch = 400 microns.

layer had continued its normal function of producing the more superficial epidermal layers which finally undergo keratinisation and desquamation. This was particularly marked in this animal (fig. 7). The appearance of such accumulated epithelial debris in a cavity whose walls consist of squamous epithelium lying on bone, is that of cholesteatoma.

In all three animals the size of the cyst was large in comparison to the original size of the graft, which approximated that of the oval window.

The cysts are represented to scale against the oval window in Diagram 1. The vertical height of the windows and cysts is easily calculated since the thickness of each section is known (10 microns) as well as the number involved. The transverse distances are easily measured with a marked eye piece in the microscope.

The cyst in animal Sk.1 had not spread greatly but had the peculiar labyrinthine extension; that in Sk.2 had eroded far superiorly and in fact its upper end was not included in the block. The site of the drum perforation is illustrated for Sk.3 and is in the lower part of the epidermoid cyst.

(b) Mucous membrane

Mucous membrane was obtained from the auditory bulla and so really represents muco-periosteum. A satisfactory graft was difficult to obtain and to manipulate because of the extreme fragility of this tissue. The grafts were implanted with the mucosal /

mucosal surface towards the middle ear.

Animal Mm.1

This animal which became suddenly ataxic on the 44th day was sacrificed on the 46th day and found to have sustained a complete necrosis of the graft (fig. 8). No remains of the graft can be identified in the oval window region which is closed by a mass of collagenous and inflammatory tissue which contained many acute and chronic inflammatory cells as well as a number of histiocytes. The inflammatory response did not involve the middle ear generally but was confined to the area of the window recess. Young loose fibroblastic tissue is invading the vestibule of the labyrinth.

Animal Mm.2

The graft has persisted as a fairly thin membrane though a small quantity of fibroblastic tissue on its superficial surface has caused its attachment to the stapedius tendon (fig. 9). The graft is somewhat everted.

The endothelial lining is detached and so more easily identifiable. In spite of its separation it remains intact as a delicate membrane so thin that its structure is difficult to define. It would appear that the layer when previously observed lining the skin grafts was somewhat thickened by hydropic swelling (fig. 10).

Animal Mm.3 /

Animal Mm.3

The graft persists as a thin membrane across the oval window. It has gained complete continuity with the mucosa of the ear and the exact extent of the original graft is impossible to define (fig. 11). Ciliated mucosa with goblet cells reached the very edge of the oval window in this particular animal.

The high power view (fig. 12) illustrates the detailed structure of the membrane. Its superficial layer consists of cuboidal or rather flattened non-ciliated cells, without any goblet cells. The thickness of the membrane is provided by a uniform layer of fairly acellular connective tissue in which blood vessels are absent. The labyrinthine surface is covered by a single layer of very thin cells which is continuous with the endosteum of the labyrinth, so delicate and so closely applied as to be hardly definable.

(c) Vein

The saphenous vein was employed and after thinning as much as possible by removal of the tunica externa and tunica media was inserted with the intima towards the middle ear.

Animal Vn.1

The graft could only be recognised as a few elastic fibres near the anterior part of the window. The graft otherwise was entirely degenerate, leaving the window unprotected except for a certain amount of newly formed collagen and a follicular collection of lymphocytes which occupied /

occupied the oval window recess. The area was diffusely infiltrated with lymphocytes as well as numbers of plasma cells and histiocytes, which in company with young fibroblasts were beginning to enter the labyrinth. Polymorphs were notably absent and the inflammatory process appeared entirely aseptic.

#### Animal Vn.2

The graft was again necrotic (fig. 13). The oval window is occupied by a mass of young collagen tissue from which a strand of more mature scar tissue extends laterally. The area near the window is infiltrated with round cells, mainly lymphocytes, among which very many elastic fibres are present (fig. 14).

A few young fibroblasts have invaded the anterior part of the oval window and remain confined to that part of the vestibule. A collection of cells enmeshed in this loose network consists almost entirely of phagocytes (fig. 15). Polymorphs were extremely few in number.

#### Animal Vn.3

A mass of mature scar tissue in which elastic fibres were identifiable occupies the anterior half of the window and the adjacent part of the middle ear (fig. 16). Round cell infiltration was much less severe than previously and confined to a relatively small area; scattered histiocytes were present and very occasional polymorphs. Mature fibrous tissue is present deep to the anterior lip of the window /

window but shows no cellular infiltration. The posterior half of the window is closed by a thin membrane (fig. 17) in which a few elastic fibres were present. The superficial surface has a covering of flattened epithelium. The extremely delicate endothelial layer such as seen already with other grafts, lines the deep surface.

(d) Conjunctiva

Palpebral conjunctiva from the lower lid was employed. The donor site healed rapidly without causing any apparent discomfort to the animal. Though thinned as much as possible, the grafts had a persistent tendency to curl and contract and were thus thicker than other grafts prior to insertion.

Animal Conj.1

The graft has herniated outwards posteriorly but has effected subsequent closure at this point by gaining attachment in the region of the facial nerve (fig. 18). This position of the graft has resulted in exposure of middle ear mucosa, still possessing normal looking cilia and many goblet cells, to the perilymph space (fig. 19). The graft retains the features of normal conjunctiva and includes several emptied vascular spaces. The superficial epithelium has been lost and there is a deposition of a small amount of loose collagen which contains large follicular collections of lymphocytes, such collections were scattered throughout the area of the oval window recess /

recess and attic.

The labyrinthine surface of the graft has an endothelial lining which fuses imperceptibly with the mucosa of the facial nerve region (fig. 20). Minute foreign bodies embedded in the graft have evoked no reaction. They are particles of stainless steel dust which were observed at the time of operation and came from the fine needles employed in manoeuvring the graft.

#### Animal Conj.2

Slight herniation of the graft posteriorly again occurred but protection of the perilymph space from the mucosa at this point was maintained by the presence of some remaining footplate.

The conjunctiva has persisted essentially unchanged (fig. 21). The number of vascular spaces is again notable. In this particular animal numbers of melanin-containing cells were observed near the edge of the graft at the time of implantation and were readily identifiable in certain of the sections. Superficially the graft has become clothed by goblet-containing middle ear mucosa over its peripheral part but at the graft's thinner central part the original flattened type of mucosa persists. The deep surface has a closely attached endothelial layer (fig. 22).

#### Animal Conj.3

The oval window and attic are filled with a mass of collagen in which the graft cannot be identified (fig. 23).

Any /

Any form of cellular infiltration of the mass is notably absent.

Although the graft is not identifiable it apparently has afforded complete oval window closure: the collagen mass makes no attempt to transgress the margins of the window, between which the endothelium is intact.

(e) Connective tissue

Subcutaneous connective tissue from the region of the skin incision was used and in the first two animals inserted as a small plug. In the last animal the graft was stretched across the oval window to provide a membrane.

Animal CT.1

The graft persists essentially unchanged without evoking any form of inflammatory cell reaction. Formation of collagen at its edges is just sufficient to secure it in the window. A small fragment of footplate persists posteriorly. The overlying mucosa is cuboidal for the most part; the deep surface is provided with a layer of endothelium against which there has been a localised deposition of finely granular material (fig. 24).

Animal CT.2

Similar features are present but rather more collagen has formed over the graft posteriorly (fig. 25). The graft has exactly the same epithelial and endothelial coats as before and there has again been a deposition of finely granular /



granular material against the endothelium (fig. 26).

Animal CT.3

In spite of the fact that the graft in this animal was inserted as a membrane across the window after an atraumatic stapedectomy, the graft cannot be recognised and is incorporated in a mass of scar tissue which contains small scattered aggregations of lymphoid cells (fig. 27).

The scar tissue has made no attempt to enter the oval window between the edges of which was a delicate endothelial lining on the graft.

(f) Adipose tissue

Adipose tissue surrounding the deepest part of the cartilaginous meatus was used and a fragment cut approximately to the size of the window. In animal Ft.2 some connective tissue was present in the graft when inserted.

Animal Ft.1

The fat graft persists but is enclosed in an envelope of mature collagen on all surfaces, thinnest on the labyrinthine side. Small fragments of footplate persist at each edge of the window as well as superficially on the graft (fig. 28). The graft envelope was clothed in columnar or cuboidal mucosa and its labyrinthine surface was endothelialised. Vascularity of the graft was notable, scattered lymphoid cells were present. No fibroblasts could be detected.

Animal Ft.2 /

Animal Ft.2

Exactly similar features are present (fig. 29). The graft and its envelope are slightly bulkier and there is an adhesion between it and the stapedius tendon and to malleus. The internal and external coverings and vascularity were as before; again no fibroblasts could be found.

Animal Ft.3

The thinnest of the adipose tissue grafts was obtained in this animal (figs. 30, 31 and 32). The graft persists as a soft, pliable-looking membrane, three to four fat cells thick. The fat cells appear entirely normal and contain a full complement of fat. The collagen envelope is correspondingly more delicate and, as in the other two, consists of fully mature tissue; no fibroblasts are present in the envelope nor between the fat cells. The envelope is covered by layers of middle ear mucosa and endothelium. The mucosa is rather flattened in type and has become partly separated during preparation of the specimen.

Vascularity was less marked; occasionally lymphocytes were scattered among the fat cells.

TABLE I

	ABNORMALITY	TIMES SEEN	GRAFT	ANIMAL
1.	Acute infection of graft area	once	mucosa	Mm.1
2.	Aseptic necrosis of graft with fibroblastic response in window recess	twice	vein vein	Vn.1 Vn.2
3.	Lymphoid response	once	conjunctiva	Conj.1
4.	Severe fibrosis	thrice	vein conjunctiva connective tissue	Vn.3 Conj.3 CT.3
5.	Bone fragments	frequently	connective tissue adipose tissue mucosa	CT.2 Ft.1 Mm.3
6.	Mucosal cyst	several	connective tissue adipose tissue	CT.3 Ft.2
7.	Adhesion to stapedius	twice	mucosa adipose tissue	Mm.2 Ft.2

## THE MIDDLE EAR

In the middle ear, any adverse response was invariably localised to the immediate area of interference, i.e. the window recess itself and the route of access via the attic. However severe any disturbance in this area, the remainder of the tympanic space was always entirely normal and the offending lesion sealed off by normal mucosa, usually ciliated columnar. Even the epidermoid cysts resulting from skin implantation were associated with otherwise normal middle ears.

To some extent certain of the associated changes have inevitably been described in the account of the grafts. Table I includes all abnormalities found in the middle ear whether previously mentioned or not.

(1)- (4) The first four processes, being intimately associated with the grafts, require no further description.

### (5) Bone fragments

Bone fragments from the stapes (as opposed to foot-plate remains between the oval window margins) were not infrequently observed in the oval window recess or adherent to the superficial surface of the graft. Their continued vitality was indicated by the presence of normal-looking osteocytes. These fragments obtain a thin coat of connective tissue but otherwise appear inert and simply gain attachment to an area of damaged or denuded epithelium such as over the divided stapedius (fig. 33). Fragments are also illustrated /

illustrated in the middle ear in figure 25 and on the middle ear surface of the graft in figure 28.

There was no evidence in these animals to suggest that such fragments stimulate excess fibrous tissue formation nor become centres of new bone formation in these situations.

(6) Mucosal cysts

The formation of mucosal cysts occurred in certain animals as the result of islands of mucosa becoming cut off by adhesions.

In the case of the animal illustrated in figure 34 (same animal as figure 27) formation of scar tissue was on a massive scale but small cysts were not infrequently observed in other animals in which minimal scarring had happened to close off a few mucosal cells in the footplate region.

The cysts were generally rounded or oval in shape and lined by normal mucosa, frequently still ciliated and containing numerous goblet cells. The mucosa was usually cuboidal or even columnar.

(7) Stapedius adhesion

Adhesion of the stapedius tendon to the graft occurred on two occasions as a result of scar tissue formation; in neither ear was any other scarring present. The stapedius muscle was normal in both and clearly capable of contraction (fig. 9 and 29).

(8) Two /

TABLE II

	ABNORMALITY	TIMES SEEN	GRAFT	ANIMAL	CAUSE
1	Acute labyrinthitis	once	mucosa	Mm.1	attic infection, graft necrosis
2	Sero-fibrinous labyrinthitis	twice	vein vein	Vn.1 Vn.2	graft failure
3	Electro- coagulation injury	thrice	vein vein skin	Vn.2 Vn.3 Sk.1	technique
4	Hydrops	five	(All the animals included in 1-2-3- above)		
5	Saccul rupture	twice	mucosa adipose	Mm.1 Ft.2	technique
6	Blood con- tamination	twice	conn.tiss mucosa	CT.3 Mm.3	technique
7	Footplate fragments	several	-	-	technique
8	Graft herniation	twice	skin conj.	Sk.2 Conj.1	technique
9	Organ of Corti damage	six	skin  mucosa vein vein vein conj.	Sk.1  Mm.1 Vn.1 Vn.3 Vn.2 Conj.1	electro-coagulat- ion injury ac. labyrinthitis graft failure electro-coag. graft failure probably electro- coag.
10	Vestibular neuro-epithe- lial damage (saccul macula only)	twice	mucosa vein	Mm.1 Vn.1	ac. labyrinthitis cautery

(8) Two negative observations are of importance:

Firstly, in no animal were any remains of gel foam found, nor any evidence of its former presence. Secondly, new bone formation from instrumental trauma in the oval window area was not observed.

#### THE INTERNAL EAR

Abnormalities observed in the internal ears of the animals were as shown in Table II.

Acute labyrinthitis and sero-fibrinous labyrinthitis were the results of graft failure

##### (1) Acute labyrinthitis

This occurred only once, with a mucosal graft and was the result of sudden late graft failure (animal Mn.1). Certain of the findings have already been mentioned. Cellular infiltration was still confined largely to the vestibule of the labyrinth; the cochlea showed severe hydrops with early infiltration of the scala vestibuli but the posterior labyrinth was not yet involved (fig. 8).

##### (2) Sero-fibrinous labyrinthitis

This was observed in two animals in which failure of the graft occurred, vein on each occasion (animals Vn.1 and Vn.2; figs. 13, 14 and 15). It did not occur in any of the animals /

animals with skin, conjunctiva, connective tissue nor adipose tissue grafts. Inadequate protection of the oval window results in invasion of the vestibule by proliferating fibroblastic tissue. A sero-fibrinous exudate develops and brings with it many round cells, particularly macrophages. As in the area of the degenerating graft itself, acute inflammatory cells are very few or absent. The process in each animal was beginning to involve the cochlea. Scanty fibroblasts, round cells and macrophages were present in the scala vestibuli and ballooning of Reissner's membrane was severe. The organ of Corti was degenerating and loss of its hair cells was in each case almost complete though the supporting elements and stria vascularis were almost unaffected. Apart from slight exudate in the perilymph space, the vestibular labyrinth was histologically normal.

(3) Electro-coagulation injury

Damage of this type to the labyrinthine capsule and the cochlea was observed in three animals (animals Sk.1, Vn.2 and Vn.3). Changes were not found in two other animals (Sk.2 and Sk.3) in which a coagulating current was similarly used to stop profuse bleeding from the divided tensor tympani. The bony lesions were similar in appearance in each case though of different extent and severity. The illustrations are all taken from one animal (Sk.1) in which all phases of the change were demonstrated in a rather widespread lesion.

It was notable that though the cochlear capsule and its contents /



contents were damaged, the utricular macula and lateral canal crista and indeed the entire posterior labyrinth contents were normal in spite of severe change in the subjacent bone (fig. 35). The irregular pattern of the lesions and the slighter degree of change around outlying vessels (giving distant foci apparently having no continuity with the central lesion unless traced in serial section) indicates that the lesion exactly followed the vascular channels.

Areas of decalcification were filled with loose fibroblastic tissue and lined for the most part by closely packed osteoblasts (fig. 36). The presence of mantles of metachromatically staining bone surrounding these areas was notable, as was the cellularity of this bone which evidently retained its osteocytes better than the surrounding bone during fixation. Many large multi-nucleated giant cells having the appearance of osteoclasts were present (fig. 37). Areas of predominantly osteoblastic and predominantly osteoclastic activity were often in close relationship with one another. The overall picture was in many respects suggestive of certain features occurring in human otosclerosis.

Lesions in the cochlea were present in ears of the three animals in which a bony lesion was found and were most severe subjacent to the area of bone change, i.e. in the lateral part of each cochlear turn. When seen in their grossest form these lesions were characterised by a necrosis of all the elements contained in the cochlear duct. The stria vascularis as well as all the structures lying on the basilar membrane were atrophic or absent. In more remote areas cellular structure survived in the stria vascularis and organ of Corti. All grades /

grades of severity were seen in each animal. In the area furthest from the point of injury, the hair cells occasionally survived though grossly abnormal. Neuro-epithelial injury of this type was not observed outside the cochlea.

(4) Hydrops

This occurred in varying degree in five animals but only as part of an extensive cochlear degeneration (acute labyrinthitis, sero-fibrinous labyrinthitis, or electric coagulation injury). Except in those cases of coagulation injury where a greater or lesser degree of necrosis of the structures within the cochlear duct had occurred it was characterised by swelling of the cells of Hensen and Deiter and occasional vacuoles in the stria vascularis, as well as the obvious bulging of Reissner's membrane.

(5) Rupture of the sacculle

The lesion was observed on two occasions (Mm.1 and Ft.2) and was in the same situation in each, directly beneath the footplate. The underlying saccular macula did not show any evidence of mechanical injury. Infection complicated the picture in one animal (fig. 8).

(6) Blood contamination

Blood cells were found in the labyrinth on two occasions.

In the first animal (CT.3) (figs. 38 and 39) the cells were /

were moderate in number and confined to the apical turn of the scala vestibuli and scala tympani. Blood was not observed entering the labyrinth at the time of the graft implantation.

In the second animal (Mm.3) (figs. 40 and 41) a considerable amount of blood contamination was known to have occurred. Blood cells are concentrated in the scala vestibuli and scala tympani of the apical and middle turns of the cochlea but were not present elsewhere in the labyrinth. The cochlea of this animal was graphically reconstructed according to the method of Schuknecht (53) for the plotting of hair cells. The spatial distribution of the blood cells can thus be accurately represented. They are seen to be present in adjacent parts of the upper two turns and concentrated in the same part of the lumen of each scala (fig. 42).

In both animals red cells only could be detected. Individual cells appeared to be normal in respect of shape, size and staining properties. No reaction to their presence was detected. Phagocytes containing haemosiderin were present on the saccule but not in the cochlea of animal Mm.3.

Blood contamination was thought to have occurred in six other animals but no evidence of its presence could be found.

#### (7) Footplate fragments

Careful examination never revealed the presence of footplate fragments inside the labyrinth even in those animals in which small pieces of bone seemed to disappear deep into the vestibule at the time of stapedectomy.

In /

In such animals spicules of bone were found adherent to the vestibular surface of the graft and bound to it by a covering of endothelium or occasionally attached to the graft by a delicate film of endothelium. The presence of these fragments was not associated with any excessive fibroblastic response nor any evidence of osteogenesis. Figures 23 and 29 are illustrative.

Footplate remains at the oval window edge were present in five animals, at the rather inaccessible posterior margin. Figures 23, 28 and 29 are examples. These fragments and remains showed no evidence of devitalisation. If multiple fragments were present in a window then bony union occurred between them but there was no tendency for new bone to grow across the window either from these marginal fragments nor from the spicules on the vestibular surface of the grafts.

#### (8) Graft herniation

In two animals exposure of middle ear mucosa to perilymph occurred due to posterior herniation of the graft as already mentioned (Sk.2 and Conj.1).

The mucosa in each case persisted unchanged; frequent goblet cells were present as well as cilia. The mucosa merged with endothelium on the deep surface of the graft and at the posterior oval window margin without making any attempt to replace it (fig. 19).

In one of these animals (Conj.1) hair cell damage was present. Cochlear plotting showed moderate numbers of hair cells to be abnormal or absent in all /

all turns. The significance of the lesion and its possible relation to the mucosa being in contact with the perilymph will be discussed.

(9) Damage to the organ of Corti

The organ of Corti was abnormal in six of the eighteen ears. The animals and any obvious associated pathology are as follows:

1. Sk.1: electro-coagulation injury
2. Mm.1: acute labyrinthitis
3. Vn.1: graft failure
4. Vn.3: electro-coagulation injury
5. Vn.2: electro-coagulation and graft failure
6. Conj.1: cause to be discussed (? graft herniation)

The characteristics of each type of lesion do not need further elaboration. As already mentioned, injury due to electro-coagulation is typified by a necrosis of all elements of the organ of Corti and of the stria vascularis, in contrast to the changes of sero-fibrinous labyrinthitis from graft failure in which the supporting elements remain relatively normal till late, hydrops is present and round cell and macrophage infiltration obvious. The changes in animal Conj.1 were not typical of either of these. The features of stimulation injury (which will be discussed later) were not present in any animal. The organ of Corti was entirely normal in the remaining 12 ears.

(10) /

(10) Vestibular neuro-epithelial damage

The vestibular labyrinth appeared notably resistant to injury. Its neuro-epithelium had not yet undergone any histological change even in cases of sero-fibrinous labyrinthitis. Only in the ear showing an acute labyrinthitis with cells streaming through a saccular rupture into the saccular endolymph space was the saccular macula degenerate. In one ear with an electro-coagulation injury (Vn.1) the saccular macula was damaged but the other vestibular sense organs were histologically intact. The vestibular neuro-epithelium thus was entirely normal in 16 ears out of 18.

INTERNAL AUDITORY MEATUS AND MENINGES

The cochlear nerve in the two animals with failed vein grafts and sero-fibrinous labyrinthitis contained occasional lymphocytes and plasma cells among their fibres.

No abnormality present otherwise in any animals.

### III. CORRELATION OF OBSERVATIONS

The histopathological findings in the middle ear, graft area and internal ear for each animal are correlated in the following summary, together with brief notes on the operation and recovery period where appropriate.

#### SKIN

##### Animal Sk.1 (figures 2, 3, 4, 35, 36 and 37)

Operation: stapes removed intact, atraumatic; graft rather small.

Convalescence: loath to walk for 2-3 days but no nystagnus nor ataxia detected; probably deaf.

Middle ear: normal.

Graft: epidermoid cyst formation with invasion of vestibule.

Oval window: clean footplate removal.

Internal ear: cautery injury of cochlea and labyrinthine capsule; saccule, utricle and canals normal.

##### Animal Sk.2 (figure 5)

Operation: traumatic footplate removal, a marginal fragment disappeared into vestibule; labyrinthine damage to be expected; graft fitted well.

Convalescence: entirely undisturbed, no ataxia nor nystagnus even on first day.

Middle ear: normal, no fibrosis.

Graft: good take, epidermoid cyst formation.

Oval window: /

Oval window: small fragment footplate remains, mucosa exposed to vestibule posteriorly.

Internal ear: sense organs normal throughout; no attempt by middle ear mucosa to invade labyrinth.

Animal Sk.3 (figures 6 and 7)

Operation: atraumatic stapes removal; graft satisfactory.

Convalescence: no vestibular symptoms whatever post-operatively.

Middle ear: normal, no fibrosis.

Graft: epidermoid cyst with attic perforation; much epithelial debris contained in cyst.

Oval window: minute fragment of footplate posteriorly.

Internal ear: entirely normal.

MUCOSA

Animal Mm.1 (figure 8)

Operation: stapes removal atraumatic, slight blood entry into labyrinth; graft fitted well.

Convalescence: entirely undisturbed till sudden severe ataxia 44th day (sacrificed 46th day).

Middle ear: } attic suppuration, young fibroblasts and  
Graft: } inflammatory cells fill attic and are  
Oval window: } entering internal ear;  
graft not seen.

Internal ear: cochlea degenerate, hydrops present;  
sacculae /



sacculle ruptured and cells entering,  
macula is degenerate; utricle and canals  
normal still apart from early cellular  
infiltration of related perilymph spaces.

Animal Mm.2 (figures 9 and 10)

Operation: atraumatic stapes removal; graft seems  
very flimsy.

Convalescence: slightly ataxic 48 hours.

Middle ear: adhesion of stapedius to graft.

Graft: good, apart from fibrotic band to  
stapedius.

Oval window: clean removal of footplate.

Internal ear: normal throughout.

Animal Mm.3 (figures 11, 12, 40, 41 and 42)

Operation: atraumatic stapes removal, blood entered  
labyrinth; graft in good position after  
considerable amount of manipulation which  
may have injured labyrinth.

Convalescence: entirely undisturbed.

Middle ear: normal; stapes fragments adherent to  
stapedius.

Graft: excellent appearance; very thin membrane.

Oval window: clear of fragments and scarring.

Internal ear: blood cells in cochlea; otherwise  
entirely normal.

VEIN

Animal Vn.1

Operation: difficult footplate removal, blood entered vestibule; graft difficult to manoeuvre into position.

Convalescence: entirely undisturbed post-operatively.

Middle ear: } attic occupied by granulations and many  
Graft: } round cells, both invading the oval  
Oval window: } window; no polymorphs; graft recognisable only as scattered elastic fibres.

Internal ear: sero-fibrinous labyrinthitis.

Animal Vn.2 (figures 13, 14 and 15)

Operation: easy removal of intact stapes; vein rather small in size.

Convalescence: entirely undisturbed.

Middle ear: } granulation tissue with many round cells  
Graft: } occupies attic and incorporates graft  
Oval window: } which is recognisable only as scattered elastic fibres; polymorphs rare.

Internal ear: sero-fibrinous labyrinthitis.

Animal Vn.3 (figures 16 and 17)

Operation: atraumatic footplate removal, graft good.

Convalescence: slight nystagmus day of operation.

Middle ear: } Excessive scar tissue present, covers  
Graft: } anterior part of graft and contains  
Oval window: } elastic fibres.

Internal ear: /

Internal ear: coagulation injury cochlear capsule and contents, vestibular sense organs normal.

### CONJUNCTIVA

#### Animal Conj.1 (figures 18, 19 and 20)

Operation: difficult footplate removal, cochlea trauma to be expected.

Convalescence: slight nystagmus evening of operation.

Middle ear: } masses of lymphoid cells in attic mucosa;  
Graft: } graft herniated posteriorly, middle ear  
Oval window: } mucosa in contact with perilymph, graft  
contains minute particles of metallic dust.

Internal ear: cochlea shows injury of moderate severity, missing and abnormal hair cells all areas, supporting elements normal; other sense organs normal; exposed middle ear mucosa shows no attempt to extend into labyrinth.

#### Animal Conj.2 (figures 21 and 22)

Operation: footplate removal in fragments, atraumatic, possibly not quite complete; melanin present in graft, good position of graft.

Convalescence: entirely undisturbed.

Middle ear: normal.

Graft: rather thick, melanin present; small mucous cyst.

Oval window: fragment footplate posteriorly.

Internal ear: normal throughout.

#### Animal Conj.3 /

Animal Conj. 3 (figure 23)

Operation: difficult footplate removal, much instrumentation in oval window, a bony fragment fell into labyrinth.

Convalescence: entirely undisturbed.

Middle ear: } attic filled by scar tissue which  
Graft: } incorporates graft, no cellular reaction,  
no definite graft remnants identifiable.

Oval window: tiny fragment footplate present, fibroblasts make no attempt to transgress oval window frontier.

Internal ear: entirely normal.

CONNECTIVE TISSUE

Animal CT.1 (figure 24)

Operation: atraumatic stapes removal; slight blood contamination of perilymph; good graft.

Convalescence: entirely undisturbed.

Middle ear: normal.

Graft: good appearance.

Oval window: small footplate remnant posteriorly.

Internal ear: entirely normal, slight granular deposit against the graft.

Animal CT.2 (figures 25 and 26)

Operation: footplate removal in fragments, fairly atraumatic; blood entered vestibule; graft fitted comfortably.

Convalescence: /

Convalescence: ataxia marked for 24 hours.

Middle ear: normal, small fragment footplate adherent to region of stapedius tendon.

Graft: good, slight excess of collagen posteriorly.

Oval window: clean removal of footplate.

Internal ear: normal throughout (slight granular deposit against graft).

Animal CT.3 (figures 27, 34, 38 and 39)

Operation: stapes removal intact, atraumatic; graft inserted as a membrane rather than a plug (no blood contamination observed).

Convalescence: entirely undisturbed.

Middle ear: } attic and oval window full of scar tissue;  
Graft: } which incorporates the graft; mucous  
                  } cyst present; ciliated columnar  
                  } epithelium covers fibrotic area.

Oval window: clean footplate removal; connective tissue has not transgressed margins of window.

Internal ear: blood cells at apex cochlea.

FAT

Animal Ft.1 (figure 28)

Operation: footplate removal after much fishing for fragments, at least one fragment lost; some blood entered labyrinth; graft comfortable fit; cochlear damage to be expected.

Convalescence: /



Convalescence: entirely undisturbed.  
Middle ear: normal.  
Graft: good appearance.  
Oval window: tiny fragments of footplate present at edges.  
Internal ear: Entirely normal.

Animal Ft.2 (figure 29)

Operation: footplate removed in fragments.  
Convalescence: entirely undisturbed.  
Middle ear: normal.  
Graft: good appearance.  
Oval window: complete footplate removal.  
Internal ear: saccular tear, otherwise normal throughout.

Animal Ft.3 (figures 30, 31 and 32)

Operation: atraumatic stapes removal; graft rather small and needed spreading.  
Convalescence: entirely undisturbed.  
Middle ear: normal.  
Graft: excellent appearance.  
Oval window: clean removal of footplate.  
Internal ear: normal.

## V. DISCUSSION AND COMMENTARY ON RESULTS

### I. CLINICAL

Although the experiment was primarily a histological study certain clinical observations are of interest.

Prior to the experiment it was expected that stapedectomy and grafting, especially in cats, would cause much vestibular disturbance. In practice nystagmus was minimal and disturbance of gait and posture absent or very transient. Acute labyrinthine destruction though also expected in view of the technical difficulty of operating on small animals did not occur.

Frequent observation of experimental animals is essential (and in the current experiment was possible) if symptoms of ataxia are to be detected: compensation in cats can be completed with great rapidity except for residual head and tail deviation which may be prolonged or even permanent and thus provide evidence of vestibular damage. Poor stability on jumping from a height of only 3-4 feet though frequently observed cannot be regarded as indicative of ataxia; it is customarily found in caged animals and those in the present experiment performed as well as their unoperated neighbours.

Subsequent examination of the cochleas revealed that severe hair cell damage was present in six ears. It is thus abundantly clear that absence of vestibular irritation cannot be regarded as being indicative of an atraumatic procedure having /

having been accomplished.

Though audiometric studies were not part of this experiment, many animals undoubtedly heard very well on simple testing.

Various authorities have illustrated by experiments on trained animals with artificially produced discrete cochlear lesions, that a very close relationship exists between the audiometric chart and the histological appearance of the organ of Corti when plotted post-mortem to show the spatial distribution of abnormal or missing hair cells. On these grounds it seems likely that 12 of these animals had completely normal cochlear function and that opening the labyrinth at the oval window can be an entirely safe procedure.

## II. THE GRAFTS

In this experiment the technique was as uniform as possible for each kind of graft for all 18 ears. A comparison can therefore be made between one graft and another as far as the associated changes are concerned.

In considering the appearances, behaviour and relative advantages of the grafts it will be recalled that certain basic criteria were recognised before the experiment began. They included availability, adaptability to middle ear conditions (especially exposure to air) and strength enough to provide a sturdy but pliable diaphragm. The presence of the graft must evoke no undesirable response from either middle or internal ear /



ear and yet it must be stable enough to resist the extension of a middle ear infection. It is evident that in addition the tissue must not be unreasonably difficult and fragile in handling (such as mucosa proved to be) nor should it be so elastic that it therefore shrinks, curls and thickens (as did conjunctiva). It should produce no secretion or debris (as did skin). The graft must provide protection to the internal ear from the moment it is inserted and hence capable of survival until the process of "taking" is completed and the graft becomes an integral part of the middle ear structure; in the process of doing so it must excite no fibroblastic nor granulomatous response (as vein especially tended to do). If it is to be utilised in human stapedectomy in cases in which the stapes suprastructure is not retained then the graft also must be suitable for incorporation in a prosthesis or capable of supporting one.

Of the tissues examined only skin is shown to be absolutely unsuitable. In all three animals hypertrophy of this type of graft occurred and resulted in the formation of an epidermoid cyst which extended far beyond the confines of the window recess as the reconstructions indicated.

One cyst had an extension into the vestibule of the labyrinth and another had perforated into the meatus. In two cases epithelial debris was beginning to collect in the cavity of the cyst, yet in all three the cyst was covered by intact, healthy middle ear mucosa. The remarkable behaviour of the skin graft results from the activity of the stratum germinatum. In virtue of /

of possessing this layer, only skin of all the grafts utilised, has cells specialised for proliferation and reproduction.

The grafts included this layer even though cut as thinly as possible. Inclusion of the layer in only a minute area would appear to be capable of giving this type of result. The original site of the basal cells in the graft approximates to the site and extent of the underlying dermis. This layer, which possesses no powers of proliferation, remained small in area but was readily identifiable in the grafts and its possession of sebaceous and ceruminous glands lends emphasis to the fact that skin must be regarded as a complex organ rather than a simple tissue.

The basal cell layer was present throughout the cyst except between the edges of the oval window: it would appear that these cells cannot proliferate in the absence of a suitable underlying matrix. The cyst wall in each case was thinnest at this point. Part of the original graft, this area is undoubtedly thinner than when inserted and consists only of the granulosa, prickle cell and corneal layers, i.e. the dying or dead layers of the epidermis. The only viable layer at this point is the underlying endothelium acquired from the labyrinth.

A further point in considering the suitability of skin for grafts in the middle ear is that the presence of a transected hair follicle implies that the graft already contains a perforation (as in fig. 6). This is clearly undesirable when attempting to bridge a gap, whether it be the oval window or the tympanic cavity. The dangers in relation to myringoplasty and /

and tympanoplasty have been stressed by Kley (54).

The behaviour of squamous epithelium in the middle ear has been the subject of comment by Ruedi (55) in a study of the pathogenesis of cholesteatoma. He has emphasised the special proliferative energy of the basal cell layer and pointed out that a cholesteatoma grows by these cells burrowing into the submucous connective tissue layer, lifting off the mucosa. This appears to have happened in these three animals: the spreading basal cell layer has burrowed into the submucosa, pushing it aside until eventually its own growing edges have been thrown back upon themselves and have united, the edges of mucosa similarly uniting over the newly formed epidermoid cyst and leaving the remainder of the middle ear cavity normal for the time being. The condition here is strikingly similar to that found in human cholesteatomata in which break down and superimposed infection have not yet occurred.

Friedmann (56) too has emphasised that a cholesteatoma is simply a cystic structure lined by keratinising squamous epithelium resting on a stroma, the keratinising layer being innermost and desquamating into the cavity to produce its contents. He stresses that it is an epithelial product of squamous epithelium and points out that keratinising squamous epithelium in ectopic sites behaves with uniformity irrespective of the site and is the originating mechanism in producing an epidermoid cyst. The same author (57) speaking of the presence of keratinising squamous epithelium in the middle ear cleft points out that such epithelium follows this regular course of behaviour /

behaviour whatever its origin. Squamous epithelium implanted into the ear of the cat does not appear to be an exception to this behaviour pattern.

In man, ectopic squamous epithelium seems to follow the same rules. Thus Escher (58) describes a patient who sustained a longitudinal fracture of the petrous temporal bone as a result of which epidermis from the meatus became implanted in the middle ear. A cholesteatoma eventually resulted. Similarly, Hall (59) has found a cholesteatoma cyst apparently resulting from implantation of squamous epithelium in the ear at the time of stapes mobilisation one year previously.

The report of Choremis et al. (60) is of interest although concerned with an area far removed from the ear. They describe the occurrence of typical cholesteatoma cysts after lumbar puncture, epithelial fragments having been pushed inwards by the needle.

That squamous epithelium cannot be permitted in the middle ear space is thus abundantly clear and has been further emphasised by Wullstein (61) as a result of his operative experiences. It must be recorded, however, that Suggitt (62) and Hall (63) have both used a Thiersch graft to cover the lateral semicircular canal fistula in cases of closed fenestration and report no untoward result. The explanation may be that from the relatively thick human skin it is easier to cut a graft which is thin enough to be devoid of any basal cells or alternately it is only in an extremely confined space such as the /

the attic of a small animal that the expanding graft becomes thrown back upon itself to produce a cyst and that in the absence of such cyst formation any keratotic debris is thrown off into the cavity of the middle ear to be evacuated via the Eustachian tube.

It is interesting to note that the first type of graft utilised by Shea to cover the opened oval window was skin (33). Perhaps realising its potential dangers, however, he very soon changed to vein.

All the other grafts are suitable in varying degree though some have certain merits and others certain disadvantages.

Mucous membrane was obtained from the auditory bulla and in this site normally has a flattened, nonciliated epithelium devoid of goblet cells. The mucosa forms a continuous layer with the underlying periosteum from which it cannot be separated; the graft therefore was really muco-periosteum. Bulla mucosa proved most difficult material to handle but when successfully transferred to the oval window provided an extremely delicate membrane. In Mm.3 the membrane was of approximately the same thickness as the membrane of the round window and very similar in character. Though attractive in appearance, such a membrane seems hardly strong enough to support a prosthesis without danger of it prolapsing into the labyrinth.

In addition it is perhaps of significance that in one of these animals the symptoms and signs of an acute labyrinthitis occurred at the 44th day even in the absence of a prosthesis on the /

the graft. Histological examination subsequently revealed the presence of an attic suppuration which had resulted in a complete necrosis of the graft. This was the only animal in which such a necrosis occurred after what appeared technically a satisfactory operation at the time. No evidence of technical error which might have predisposed to this result was apparent.

The corresponding source of supply of this type of graft in man would be muco-periosteum from the mastoid air cells, where it is approximately of the same texture. Muco-periosteum from the maxillary sinus though much thicker and tougher (and so perhaps more suitable) would appear aesthetically rather unpleasant and has the serious disadvantage of greater potential risk of introducing infection. Nevertheless, Kley in unpublished work quoted by Wullstein (64) has found this type of mucosa to be the most practical for use in tympanoplasty when attempting to graft areas devoid of mucosa. He has found it far superior to oral mucosa and conjunctiva for this purpose (65). But at the oval window a mucosal graft appears too hazardous: if thin enough, handling is difficult; it may have a tendency to break down in the face of infection; it is probably too feeble to support a prosthesis for long; the necessity to obtain it through or from a potentially infected cavity renders it quite unacceptable for use at the oval window in human stapedectomy.

The results after vein grafting the oval window were the most unsatisfactory of the whole series though an error in technique and resultant injury from improper use of the electro- /

electro-coagulation may have been partly to blame in one animal, in which excessive fibrosis occurred. In the other two animals total failure of the grafts resulted, the only recognisable feature of the graft being persistent elastic fibres in a mass of granulomatous tissue.

The absence or rarity of polymorphs suggests that the process was not an inflammatory one and the absence of any symptoms of vestibular failure indicates that destruction was slow enough for compensation to occur. The type of inflammatory response, viz. lymphocytes, plasma cells, histiocytes and active macrophages, is that invoked by necrotic tissue at any site. The widespread distribution of these cells suggests that the cytotoxic agents responsible were diffused widely through the areas of the attic, internal ear and slightly in the substance of the eighth nerve.

The actual cause of this overwhelming overgrowth of young fibroblastic tissue and graft destruction is difficult to decide. It is now known that a similar type of reaction occasionally occurs after an apparently perfectly satisfactory and atraumatic human stapedectomy and graft, the effect upon the internal ear being as disastrous as in these animals. It is apparently not confined to vein-grafted windows and perhaps represents an inflammatory granulomatous response to trauma with exudation and exuberant repair such as may follow any surgical or traumatic injury elsewhere.

The initiating mechanism appears to have been a necrosis of the graft, almost certainly on a nutritional basis. In this experiment /



experiment vein probably had the greatest nutritional needs of all the grafts. It is likely that in these animals the nutritional area provided by the oval window and adjacent de-epithelialised bed to the comparatively bulky graft was insufficient. Wider de-epithelialisation may have helped maintain nutrition, which initially must be by diffusion across this interface until vessels have become established. Failure at this stage could result in the state of affairs seen in these animals.

Bellucci and Wolff (42), who have been responsible for the only comprehensive studies to date on experimental vein grafting, obtained essentially similar results though the fibroblastic activity was much more advanced and more extensive. It would appear that in certain of their animals the technique was not entirely satisfactory but their observations are important, especially in view of the paucity of other contributions.

During the course of a variety of oval window procedures they carried out vein grafting on nine stapedectomised cats. One animal died at three days and in their paper the graft is illustrated as a bulky amorphous plug rather than a membrane. They report early degeneration of the cochlea.

Two of their animals were sacrificed at 10 days. In neither was the vein recognisable amid the mass of fibroblastic tissue which already filled the area. The type of cellular response in one of these ears, however, suggested that an acute inflammatory element could not be ruled out.



Six of their animals survived between five weeks and five months and in each of these was a severe fibroblastic response involving the attic and oval window. The grafts were only recognisable from the presence of scattered elastic fibres. The reaction involved the internal ear very severely in every case. In one animal the number of polymorphs suggested an inflammatory element though in the remaining five the result was similar to that in the present experiment. The fibroblastic process was generally much more extensive and more mature, but in one ear the sense organs were described as good apart from damage to the basal cochlear turn.

They searched carefully but unsuccessfully for fragments of stapes or bone dust which might have initiated the response. They also searched carefully for polymorphs particularly in the blood vessels of the part but were forced to the conclusion that the process was entirely an aseptic necrosis and were not able to submit any suggestion about the initiating mechanism.

It is clear from the illustrations which accompany their paper, that the grafts were introduced too deeply into the window. Perhaps their results indicate that on no account must the graft be inserted beyond the margin of the window.

Myers et al. (41) in their experiment already referred to were able to examine vein grafts from the ears of five of their thirteen cats, the survival times of which varied between eight and 106 days. Unfortunately, the temporal bones were not sectioned as a whole, the grafts simply being dissected out post-mortem. It was thus not possible to study the response of /

of related parts. They report that the vein grafts had become much thicker due to invasion by collagenous tissue, many capillaries were present and re-vascularisation was considerable. The middle ear surface was covered by cuboidal epithelium.

Animal work has thus been somewhat inadequate and unsatisfactory and in fact has served mainly to illustrate the results of failure without providing much information about the appearance of a successful graft. Certain observations however have been possible on human ears re-opened after an unsatisfactory result. Shea has examined several ears in which a dislocation of the polythene strut occurred and found the vein to be lying in the position in which it had been left in the window, entirely free from strands of scar tissue and quite thin. It was paler than the surrounding muco-periosteum from which it could be easily distinguished. The graft was quite elastic and freely movable over the fenestra which could easily be seen through it (66).

He has also described the histological appearances of two vein grafts recovered from ears operated upon 18 months previously (67) (reason for re-exploration and removal of the graft not stated). He found the graft to consist of a thin membrane of connective tissue containing very many elastic fibres, the membrane being covered by a layer of mucosa.

Two complications which occurred in the current experiment, viz. aseptic necrosis of the graft and excessive fibrosis, are undoubtedly responsible for a proportion of human failures.

The /

The frequency of aseptic graft necrosis is difficult to estimate as the experimental evidence shows that it must inevitably result in a completely deaf ear and few of these have been explored. In practice, it probably accounts for a significant proportion of "dead ears", i.e. cases with total loss of cochlear function, the frequency of which Shambaugh has put as high as 11% (68), and is perhaps responsible for the majority of those which occur relatively late in convalescence in patients apparently progressing normally. This view tends to be supported by observations made in those few ears which have been re-explored within an hour or two of the onset of cochlear type hearing loss and in which exuberant granulation tissue has been found filling the oval window recess beneath a layer of intact mucosa. The oval window remains closed, as in these animals, by the mucosa and fibroblastic tissue whilst the products of graft necrosis diffuse widely into the internal ear. This type of graft behaviour does not appear to be confined to vein (69).

Failure due to severe fibrosis around the graft is again of uncertain frequency. Shea regards this as infrequent, having observed it only 45 times in a series of 1500 patients (3%) and states that in the last 200 cases with a follow-up of 3 months or more a middle ear deafness persisted in only 6% of cases in excess of 10 db. whatever the cause (70, 71). There must be few otologists who can claim anything like his success. He has expressed the view that the most important factors predisposing to excessive fibrosis are exudation and bleeding (72). /

(72). This opinion, however, is open to question: haemotympanum and exudation are of frequent occurrence both in animal surgery and human operations involving the middle ear and oval window recess and can be easily recognised by examination of the drum. Permanent damage is unusual, the middle ear normally evacuating itself without difficulty providing the Eustachian tube is functioning and the middle ear mucosa healthy. He has also blamed excess scarring on the fact that in a narrow window recess, the small distance between the facial nerve and promontory causes the vein to become crowded around the polythene tubing so that the healing reaction in some patients then results in excess granulation formation (66).

Excessive scarring is more likely to be the result of retention of clot or exudate in areas where the mucosa has been damaged during difficult instrumentation or denuded excessively in preparing a bed for the graft.

Examination of conjunctiva in the current experiment showed its tendency to shrink and curl to be most disadvantageous features. Prior to insertion, contraction caused a reasonably sized thin graft to become small and thick and the associated curling was particularly troublesome during positioning over the window. The increased difficulty of instrumentation entails risk of mucosal damage and fibrosis as suggested.

Fibrosis was severe in one animal but though it incorporated the graft, there was no tendency for it to cross the oval window threshold. Though poor as a graft, complete oval window protection was afforded. In a second animal the fibrous tissue was /

was less excessive but contained several large aggregates of lymphoid cells. In this animal, however, fibrosis did not incorporate the graft and a plane of separation was clearly identifiable due to the partial persistence of the original conjunctival epithelium. This particular graft contained fragments of fine metallic dust which had evoked no reaction in the graft; the graft had the appearance of normal conjunctiva. Herniation of the graft posteriorly was a probable result of the difficulty in handling.

The most satisfactory result was that obtained in animal Conj.2. Though rather thick, the graft appeared to have undergone no change since insertion and in fact still contained pigment cells at its edge which were noted at the time of implantation. This part of the graft was covered by epithelium which was clearly of middle ear origin, richly endowed with goblet cells. Away from this area the graft was covered in flattened epithelium which presented a fairly sharply defined junction with the goblet cells, was probably the original epithelium and apparently perfectly acceptable.

The behaviour of conjunctiva has been studied by Wullstein (64) in his search for a practical type of mucosa for use in tympanoplasty but there have been no reports of work concerning its use across the oval window. Wullstein chose it for evaluation because it appeared to have all the qualities desirable for a middle ear graft: it is well adapted for exposure to air, produces no secretion and possesses no appendages. He, too, found that as a result of having a rich elastic /

elastic network shrinkage was troublesome and it is for this same reason that it is not entirely suitable for use in the oval window.

Connective tissue had the virtues of being easily obtained and simple to handle.

The plug of tissue persisted unchanged in two of the animals, achieving adequate protection and providing a pliable membrane which appeared substantial enough to support or be incorporated in a prosthesis.

Occurrence of fibroblastic tissue was minimal in these animals and served only to provide secure attachment of the graft to the margins of the oval window. There was no evidence to suggest that the presence of connective tissue in the window stimulated excessive scar tissue in the region.

In both cases the graft was provided with a covering of normal middle ear mucosa. Excessive scarring was notable in another animal and is of added interest because the stapes removal was particularly easy, no mucosal damage was thought to have occurred and the graft was spread out as a thin membrane across the window instead of being inserted as a plug.

It is difficult to explain this occurrence and it seems that a fibrotic result can in fact occur after a perfectly satisfactory implantation. It would probably be wrong to associate it with the type of graft in view of the good result with the other two bulkier grafts and is probably a manifestation of the excessive repair reaction which sometimes occurs after /

after injury.

In all three animals the grafts gained a covering of middle ear mucosa, cuboidal or columnar, with or without goblet cells depending in the particular type of epithelium present in the adjacent part of the middle ear.

Although there have been no other animal studies whatever on the behaviour of connective tissue in this region it has now been extensively used as an oval window graft in the human with good results that have been well maintained (35).

Adipose tissue too, possesses the advantage of being readily obtainable, easy to handle and can easily be used with a prosthesis. Like connective tissue it is not necessary to de-epithelialise beyond the margins of the oval window to provide a satisfactory bed and the risk of fibrosis is correspondingly diminished.

In addition, this type of graft was found to have the special advantage of adapting itself particularly well to the shape of the window. In one animal in which the graft was rather small, it was observed to float on the surface of the perilymph and could be teased out to form a membrane which filled the defect completely. The final appearance here was particularly attractive. It has been possible to utilise these features in human operations several times since and, in fact, it appears advantageous to have a graft which is slightly small and to tease it out to the edges of the window. The natural bouyancy of the graft, combined with the fact that it is usually /



usually incorporated in a steel pin which is already suspended from the incus, eliminates any danger of the graft becoming displaced into the vestibule.

Unlike connective tissue, fat undergoes modification after being inserted in the oval window. The most important change which was obvious in all three animals was the acquisition of a delicate connective tissue envelope by the graft to cover both its internal and external surface as well as that part through which attachment was gained to the edges of the window.

In spite of the presence of this envelope, formation of connective tissue or scar tissue in adjacent areas was noticeably absent and there was no heaping up of fibroblastic tissue upon the graft in any animal. There appear to be three possible explanations of the origin of this connective tissue envelope.

First, the graft at the time of insertion may in fact have been a mixture of adipose and connective tissue together. In practice it is not always possible to differentiate the tissues with lower powers of magnification. A few superficially situated fat cells on a predominantly connective tissue graft can glisten as though the tissue is purely adipose in type. The opposite type of mistake can also occur: histological examination of tissues for use as possible controls showed a "connective tissue" graft to consist entirely of fat except in its most superficial parts. Routine use of the highest power of the operating microscope (40 diameters) does not prevent all cases of mistaken identity.

Second, the envelope may have been obtained by organisation of /



of exudate or blood clot lying immediately adjacent to the graft, though it is difficult to explain the fairly uniform thickness of the layer on each aspect of all three grafts. Such an explanation may have been acceptable if the layer was confined, or at least greater, on the middle ear surface, where such a collection of clot or exudate might reasonably be expected to be greatest. The appearances of other types of graft suggest that when this occurs the amount of tissue so formed is much more massive, tending to incorporate relatively insignificant graft remnants rather than simply forming a protective envelope for it, before receiving its final coat of mucosa.

A third explanation is felt to be more acceptable. Fat is a relatively specialised kind of tissue and it is well recognised that in certain conditions reversion to a more primitive form can occur; adipose tissue in the more usual body sites is not in a static condition but rather in a state of balance. Cells become specialised to accommodate fat if necessary but are just as capable of de-differentiating to ordinary connective tissue. It is not inconceivable that grafted adipose tissue exposed for a time to certain adverse and abnormal conditions, which temporary disturbance of blood supply does not mitigate, reacts in this way in its more exposed peripheral parts.

It may be argued that, given time, complete fibrous replacement of the graft will occur. Only a long-term experiment can finally determine this but meantime this assumption is resisted on the following grounds:

(1) /

(1) There is no evidence on examining these grafts that the process is still continuing. The envelope consists throughout of completely mature fibrous tissue; young fibroblasts are notably absent both from the deep surface of the envelope and from the fatty centre itself. (2) Throughout the interior, the adipose tissue cells all contain a full complement of fat and conditions for their continued well-being appear adequate, especially in respect of blood supply. Vascularity in all these grafts was notable and it is possible that most of the de-differentiation of the adipose tissue occurred before vascularity was secured. (3) It must also be remembered that fibrous replacement is not the routine fate of specialised tissues exposed to chronic injury or inflammation. Pathologists are familiar with the fact that though lymph glands or pancreatic tissue for example may undergo fibrotic replacement, this in turn becomes replaced by adipose tissue.

Whatever the origin of the envelope, its presence actually enhances the suitability of adipose tissue and it probably aids the incorporation of a prosthesis. Adipose tissue alone forms a poor barrier to spread of infection and in the unlined state would appear to provide an unsatisfactory surface for endothelium and for epithelium especially for conditions prevailing in the middle ear. The development of the envelope, whilst in no way interfering with the moulding of the graft to the shape of the window, strengthens what might otherwise be too flimsy and unreliable a structure. The delicate architecture of the surviving /

surviving fat-containing cells together with the fact that at body temperature their contents have fluid consistency, combine to make this a very pliable graft.

In summary, from examination of various types of graft, it is apparent that the different tissues all persist as readily identifiable entities though some undergoing certain modifications. Skin undergoes proliferation and cystic hypertrophy; mucosa remains apparently unchanged; vein undergoes marked thinning though its elastic elements persist and may be the only recognisable element present in the presence of excessive fibrosis; conjunctiva shrinks and thickens; connective tissue persists unchanged; fat acquires a connective tissue envelope.

Though these are the findings at a relatively short time (viz. approximately five months), there appears to be no evidence whatever of any process which suggests complete replacement of these grafts by fibrous tissue, nor need such a process be expected once the graft has survived the initial period after transfer. The suggestion of Kos (73) that any type of graft eventually becomes simple scar tissue is purely conjectural and has at present no experimental basis. It is, however, quite possible that the special method of dealing with the vein in his operation of "vein plug stapedioplasty" does produce this result (74). This being his aim, it is difficult to see how the operation or even the instruments vary at all from the technique discarded by Schuknecht 20 months ago in favour of the present method of fat graft and pre-formed steel pin prosthesis (35). /

(35).

Of all the tissues examined, adipose tissue gave the most constantly satisfactory results. On the evidence so far submitted it would appear to be the most suitable type of tissue to replace the stapes footplate provided no undesirable effects in the middle or internal ear are shown to be caused by its presence in the oval window.

As a graft it can readily be used with a prosthesis and the experimental evidence indicates the following advantages:

- (a) it is readily available; (b) it is easy to handle and insert; (c) it does not require a de-epithelialised bed, the exposed edge of the stapedio-vestibular joint is adequate and the risk of excessive scarring is correspondingly diminished;
- (d) it tends to float on the perilymph surface and thereby spread out and mould exactly to the shape of the window;
- (e) the envelope which subsequently forms ensures the permanency of this "moulding", in no way interferes with the pliability of the graft, yet strengthens it physically and increases the resistance to any spread of infection from the middle ear; and (f) the persistence of any fat cells, the evidence being that most of them do persist, further adds to the flexibility of the graft.

### III. THE MIDDLE EAR

To some extent the middle ear responses have already been discussed /

discussed since they are inevitably closely connected with the incorporation of the graft itself into the oval window.

However good or bad the condition in the oval window area, it was notable that any pathology was always confined to this area and was walled off by a layer of mucosa. The presence of an epidermoid cyst was entirely ignored by the middle ear. Even in the one animal in which an acute infective necrosis of the graft occurred, the remainder of the ear was normal and there was no suggestion that infection had arisen via the Eustachian tube lumen or submucosa. The strict localisation of the inflammatory response suggested that the infection had either been introduced with the graft and had lain dormant, or more likely in view of the delay, that a haematogenous infection had occurred in an area of impaired vitality.

It is noteworthy that in the two animals with an aseptic graft necrosis the area was also completely closed off by healthy mucosa which in fact afforded complete oval window protection, albeit ineffective because of the widespread diffusion of toxic products from the underlying dying graft.

The cellular response towards one of the conjunctival grafts was of interest in that it was purely lymphocytic in character and though not associated with graft destruction nor the presence of macrophages was reminiscent of the response in aseptic graft necrosis. This response was seen to a minor degree in animals with other types of graft.

Lymphoid /

Lymphoid follicles are frequently present in the middle ear submucosa of perfectly healthy cats but are usually so insignificant as to escape notice. In this animal however the follicles were prominent in size and number and maximum in the area around the graft; vast numbers of lymphocytes in follicular collections were concentrated over the graft itself and spilt over into the bulla. Yet there was no tendency for the cells to infiltrate the graft itself.

Though it is generally accepted that lymphocytes are primarily concerned with antibody production it appears unlikely that an autogenous graft should incite an antigenic response. Indeed, the entire field of transplantation surgery in the past has been limited by the fact that an autogenous graft (or one from a truly identical twin) is the only type which does not evoke an adverse host reaction.

The phenomenon here must nevertheless be related to the graft and it represents perhaps a reaction to damaged cells included in the transplant.

The development of excessive fibrosis in the middle ear has to some extent already been discussed and Shea's opinions on its occurrence with vein grafts have been recorded. In this experiment it occurred in three animals, with vein, conjunctiva and connective tissue grafts. It cannot be related to the type of graft though in fact was not observed with skin, mucosa nor adipose tissue.

As already indicated it is the result mainly of mucosal damage /

damage and so may occur during difficult instrumentation or excessive mucosal denudation when preparing a bed. With those grafts which can be inserted between the actual edges of the window (connective tissue and adipose tissue), the latter cause is correspondingly reduced in importance. It is perhaps not without significance that Bellucci and Wolff in the description of their experimental technique state that the oval window recess was denuded of epithelium before inserting their grafts, though they make no attempt to correlate this with the fibrotic end-result which occurred so regularly in the middle ears of their animals.

When fibrosis occurred it was usually severe and completely incorporated the graft. It was notable that however dense and extensive the fibrosis in the middle ear it showed no inclination to transgress the original position of the graft and so invade the labyrinth. This distribution has recently been commented upon by Hohmann (75) in a repeat series of experiments and he too has reached the conclusion that fibrosis results primarily from excessive instrumentation and mucosal injury.

There was no evidence in the current experiment that bone fragments in contact with soft tissue in the middle ear initiate excessive formation of fibrous tissue in the oval window recess. Bone chips were observed on several occasions but their presence appeared entirely innocuous. They become surrounded by a thin fibrous sheath and gain attachment to any convenient de-epithelialised area, frequently the stump of the divided /



divided stapedius and having done so cause no further change.

Strands of scar tissue which are prone to develop by organisation of clot or exudate between any raw areas and were responsible for two conditions: the development of mucous cysts and adhesion of stapedius to the graft.

Mucous cyst formation was noted several times and simply results from islands of goblet-containing mucosa becoming cut off from the remainder of the middle ear space. In the cat, in contrast to man, goblet cells often occur in great numbers in all parts of the middle ear space (except the bulla) and are not infrequently seen in the ciliated columnar epithelium at the very edge of the oval window. The oval or rounded shape of the cysts and the fact that their lining epithelium was in no way atrophic was suggestive of the absence of any degree of tension within them. Their epithelium appeared to be still ciliated and their goblet cells still productive, the dense staining of the cyst contents and their granular, foamy character gave the appearance of concentration.

It is likely that a certain stage will eventually be reached at which these cysts will become static or degenerate but in any case the paucity of goblet cells in this area in man entails that they are unlikely to be a problem in human surgery.

Adhesion of the stapedius tendon to the graft, however, is clearly undesirable. There was no associated fibrotic degeneration of the muscle fibres and such an adhesion could doubtless /



doubtless lead to traction on the graft and impair its mobility without necessarily providing the normal protective action of the stapedial reflex.

It can occur even when scar formation is minimal and may be the only scarring present. Division of the stapedius tendon results in its prolapse, the extremity readily adheres to any un-epithelialised surface and the nearest is usually the graft itself at this stage. The possibility of this complication was appreciated during the course of the experiment and therefore in the later operations the tendon was always divided as near to the pyramid as possible. It would appear advisable to follow the same practice in human stapedectomy.

In the majority of animals none of these possible middle ear conditions occurred. Generally the graft becomes covered with cuboidal or columnar epithelium depending on the type of mucosa which happens to be present in adjacent areas.

Certain grafts already present a surface to the middle ear which is acceptable without modification: a mucosal graft, as would be expected, present an acceptable surface; vein intima is changed very little; conjunctiva appeared to retain its original epithelium except at the edges (where it was possibly damaged anyway).

In every experiment the graft was supported in position by a fragment of gel foam but in no animal was any evidence of its former presence recognisable. In those animals in which an excessive /

excessive amount of scar tissue occurred, the gel foam was no doubt incorporated in the antecedent blood clot but can hardly be considered a prime factor in the sequence of events.

The time at which gel foam disappears varies according to different authors. Bellucci and Wolff in their paper already referred to (42) found gel foam to be unchanged at 3 days; lysis was beginning at 13 days. It is of incidental interest to note that at just over two months they were still able to detect lysed particles in the vestibule in a case in which gel foam had been inserted to close the window without an actual graft.

Withers (45) reported that gel foam was completely evacuated from the ear at six weeks in his experimental animals. The animals in the current experiment all had survival times in excess of this. It can be said that gel foam remains long enough to support the graft during its uncertain early days and satisfactorily absorbs within a few weeks to leave no trace.

The occurrence of new bone formation around the traumatised footplate and oval window recess of animals has been described by Schuknecht (76) and by Bellucci (77) but the phenomenon was not encountered in this experiment. They state that in experimental animals not only can fractures of the crura and footplate unite but that ankylosis of the footplate to the margin of the window can result. It is recognised that human stapedial fractures readily unite if in good position but refixation of a mobilised footplate is usually attributed to refixation /

refixation through the otosclerotic focus. This is apparently not necessarily the case.

#### IV. THE INTERNAL EAR

Whatever the appearance of the graft and whatever the response in the middle ear, the prime essential is that an adequate seal must be provided across the oval window to afford immediate and lasting protection to the sense organs of the internal ear.

It would appear that all the six tissues examined are capable of providing this, even skin which for other reasons was entirely unsuitable. In only one animal did protection prove to be temporary and resulted in an acute labyrinthitis when the rather flimsy graft (mucosa) broke down. In a favourable case, any type of graft becomes lined by endothelium and recent further work by Schuknecht (78) on the behaviour of adipose tissue grafts has shown this layer to be complete in animals sacrificed at the tenth day. There is no reason to suspect that this time is any different for other types of graft though examination of the connective tissue grafts especially showed the persistence of a granular proteinous deposit against the graft which probably represents the response by the perilymph to a foreign tissue, in the interval between implantation and endothelialisation.

Although the sense organs of the internal ear were entirely /

entirely normal in 12 ears and damage in another 3 was clearly due to technical fault involving the use of electro-coagulation, several conditions were recognised which clearly have a close relevance to human work and of these, sero-fibrinous labyrinthitis is the most important. It would be wrong to attempt to correlate the various conditions too closely with the type of graft employed though the consistently poor result with vein was surprising in view of the favourable human results which are now well known. Only the connective tissue and adipose tissue experiments were entirely unassociated with internal ear complications relative to the graft.

Whatever the responsible factor in animals found to have internal ear damage, the severity of cochlear damage and mildness or absence of vestibular damage was notable. The appearances in sero-fibrinous labyrinthitis were no exception to this observation and the toughness of the vestibular neuro-epithelium to histological damage is notably emphasised in these animals. It is very significant that these animals were never ataxic. The condition is the result of graft necrosis and the response is mainly caused by diffusion of cell breakdown products. The oval window remains closed by the fibroblastic reaction and the intact middle ear mucosa as already noted.

This may well represent the pathology in those cases of sudden cochlear failure without vertigo, which occur in human patients after an apparently satisfactory grafting operation. The oval window diaphragm appears to be functioning satisfactorily until the sudden release of toxic products causes havoc /

havoc in that part of the internal ear which is most sensitive and most accessible from the oval window, i.e. the basal turn of the cochlea. This is the process which is probably occurring under cover of the area of exuberant repair noted in those few patients whose ears have been urgently re-explored within a few hours of cochlear failure.

Though this explanation may be applicable to those cases of human failure which occur relatively early in convalescence it is not satisfactory for those cases of acute cochlear failure which occur late. The observation of Singleton and Schuknecht (40) is probably relevant to these cases. In the course of their animal investigation into the effect of trauma around the stapes footplate, they showed that a minute fistula between middle ear and labyrinth can persist for as long as 290 days without producing symptoms and without damage to the internal ear. They illustrate this in their paper by a section which shows a strand of polymorphs beginning to enter the vestibule. The fistula was just large enough to admit polymorphs yet had failed to close spontaneously. The appearances in animals in the current experiment with labyrinthine degeneration suggests that damaging factors, whether infective or toxic, entering via the oval window may be limited to the cochlea in their effect especially if invasion is slow and spread prevented by fibroblastic deposition.

There is no reason why fistulas should not persist for 290 days in human oval window particularly if the graft is not in perfect apposition. From the article of Singleton and Schuknecht it is evident that the deficiency need be very small indeed /

indeed and perhaps difficult to see with the ordinary operating microscope especially with the view partly obscured by the incus. The risk is perhaps least with grafts such as adipose tissue where moulding tends to give an exact fit to the window margin but the risk is present in almost any stapes procedure and has been personally observed at re-exploration after an ordinary mobilisation which resulted in a "dead ear", again without vertigo.

A further significant cause of internal ear damage was the improper use of electro-coagulation. This was used on the divided tensor tympani muscle when bleeding from it could not be otherwise controlled and sometimes resulted in damage to the underlying structures, usually the cochlea. (This deficiency in technique was not thought to be a factor in the development of excessive fibrosis in certain animals: a severe electro-coagulation injury was inflicted upon two skin grafted animals (Sk.1 and Sk.2), in which scar formation was entirely absent. In contrast much scarring occurred in other animals in which no electro-coagulation was used.) Shea (34) has drawn attention to the dangers of using the electro-coagulation needle in the region of the middle ear and the possibilities have been demonstrated in three of these animals.

The injury as it affects the cochlear contents is easily recognised from other types of injury because all elements of the stria vascularis and organ of Corti are equally affected, maximally in those parts of each cochlear turn nearest to the point of application. In those with the grossest injury no trace /

trace of the organ of Corti may remain on the basilar membrane yet in certain of these perfectly normal looking nerve fibres were present. They clearly could not be surviving afferent hair cell fibres in view of the very severe necrosis inflicted and must thus represent fibres which had newly grown in. The only fibres capable of this would be efferents, these fibres must therefore be those first described by Rasmussen (79, 80) and called by him the "olivo-cochlear tract". The work of Portmann (81) and Schuknecht and Woellner (82) has indicated that they probably normally end on the inner hair cells. They are cholinergic in type and probably inhibitory according to Churchill and Schuknecht (83); their apparent survival posed a difficult problem in this experiment until their true nature was realised.

These accidental electro-coagulation injuries produced an interesting change in the labyrinthine capsule. The widened vascular spaces containing loose fibroblastic tissue, the frequency of giant cells having the appearance of osteoclasts and the intensive osteoblastic activity and mantling of the spaces are features which give a close resemblance to active otosclerosis. These sections were shown to a pathologist particularly interested in the histopathology of this peculiarly human disorder and it was agreed that the appearances had much in common. In both conditions the picture is one of bone removal and repair by replacement; in neither is the need for bone removal obvious on histological grounds but presumably the bone is in some way abnormal. The passage of an electric current /



current may interfere with vital enzyme systems and thereby disturb the metabolic processes though no clue to the abnormality could be detected in the surrounding bone areas except possibly a diminution in the osteocyte population.

The process in these animals and in human otosclerosis is perhaps a non-specific type of repair which can be in response to a variety of damaging factors or inborn abnormalities.

Bellucci and Wolff (84) in a paper which came to hand after these interesting similarities had been noted, have also drawn attention to the otosclerosis-like lesions resulting from electro-coagulation injury. They have attempted to place the various types of change in chronological order as follows (i) vasodilatation with a sleeve of decalcification around the vessel; (ii) a chemical change in the surrounding bone as evidenced by the metachromatic staining of the mantles; (iii) widening of the decalcified zone and fibrotic replacement; and (iv) osteoclast formation. They do not attempt to correlate osteoblast activity with any stage but the suggestion is that it occurs after the formation of the mantles; neither do they submit reasons for placing the various phases of activity in the above order and it seems odd that osteoclast formation should be their last stage.

They also raise the possibility that these changes represent a non-specific type of response by bone which may perhaps be initiated by widely different agents. While not claiming to have produced histological otosclerosis they believe that they may have found one of the basic factors responsible /



responsible for producing such lesions, viz: a disturbance to the vascular supply of the otic capsule. They suggest that another agent, yet unknown, may be capable of producing the same result in the human otic capsule and express the hope of further work based on this finding.

Labyrinthine hydrops was observed only as part of a severe labyrinthine lesion. Hohmann (75) has stated that it is the earliest and commonest reaction found after various stapes footplate procedures. He found it to be present in 17% of procedures involving footplate fracture and in 26% of stapedectomies. The animals he studied had been operated upon by various persons and his stapedectomised animals consisted of those of the present experiment and a number of fat-grafted animals of a subsequent experiment. It is not clear whether his figure of 26% includes animals in which a sero-fibrinous labyrinthitis occurred, but the figure of 17% after a less radical procedure is of interest.

He has also described an opposite type of change which he calls hypotonic atrophy. It is characterised in animals by a shrunken appearance of the contents of the scala media, depression of Reissner's membrane, flattening of the tentorium on to the organ of Corti and severe shrinking of the saccule. The disorder is a rare complication of footplate fracture and stapedectomy and was not observed in this experiment.

Damage to the membranous labyrinth was found in two animals. In both the saccule was involved and in both the injury was directly beneath the centre of the oval window; it almost certainly resulted from footplate instrumentation. The close proximity of the saccule to the footplate renders it especially vulnerable to injury at the time when the footplate is penetrated prior to removal of the fragments. Injury would probably be more frequent but for the tendency of the membranous labyrinth to yield and fall away as can be seen in human lateral semicircular canal fenestration.

Of the animals with saccular tear, acute infection complicated one case but in the other the neuro-epithelium was normal throughout the labyrinth. Singleton and Schuknecht (40) in contrast found a stimulation injury of the cochlea in all but one of eight ears in which they discovered a lesion of the saccule and they found a lesion of the saccular membrane in every ear in which a fenestration of the footplate or annular ligament was made. It must be emphasised however that their experiment was directly concerned with the results of carefully inflicted trauma aimed at the stapes, whereas the current experiment was done as atraumatically as possible.

Apart from the hydrodynamic disturbance occurring in footplate manipulation and capable of producing damage at a distance, it is surprising that direct instrumental injury is not in fact more common than the present experiment suggests. The close proximity of certain vital structures to the oval window even in the larger human ear puts these parts in considerable /

considerable hazard and the possibility of instrumental damage must constantly be borne in mind. Anson and Bast (85) have recently stressed the close relationship of the structures in this region in a careful and most important anatomical study.

The following measurements are of special interest:

Anterior part of oval window to saccule 0.75 to 1.6 mm.

Anterior part of oval window to internal meatus 1.75 mm.

Upper part of oval window to utricle 0.5 to 1.6 mm.

Anterior part of footplate to proximal end of cochlear duct 0.3 mm.

Although the distances from the oval window to utricle, saccule and cochlear duct are extremely small it is notable that in the animals there was never any tendency for adhesions to form between them and the graft, whether the latter was satisfactory or not. It is possible that removal of a graft in the course of a human revision operation may not be so hazardous as Shea has suggested (70).

Blood was present in the internal ear of two animals; the blood cells remained confined to the perilymph space and were found in the scala vestibuli and scala tympani. Blood was not found in the ears of five other animals in which contamination was thought to have occurred.

No white cells or thrombocytes could be identified but the red cells appeared perfectly normal in shape, size and staining characteristics even 18 weeks after operation. In both animals blood cells were present in the adjacent parts of the scalae of adjacent /

adjacent cochlear turns; they appear to behave as entirely inert foreign bodies floating about in the perilymph and going wherever fluid currents and gravitational forces take them.

The ordinary life of the cat's red blood cell is probably comparable to that of the human, which has a maximum of about 100 days in the circulation. Thus they survive longer in the internal ear and persist in a good state of preservation when ~~so~~ protected from the violent buffetings to which they are properly heir.

Schuknecht and Davidson (86) found many red cells in the cochlea five months after head injury (i.e. slightly longer than in these two animals) but the exact time which cells can persist remains unknown. Removal of the cells is undertaken by phagocytes but their paucity indicates that clearance is slow in the extreme when the contamination is of any magnitude. Phagocytes with contained red cells and haemosiderin could be found only in one of the two animals in this experiment. There was no evidence in the current experiment that blood or its break down products had any adverse effect on the internal ear. Hohmann (87) in a recent animal study in which he injected blood into the labyrinth has reached a similar conclusion.

House (88) has expressed the same views in relation to human stapedectomy but Shambaugh (68) in comparison found a relationship between bleeding and cochlear loss in human work. In view of the experimental evidence, however, it would appear that the relationship is an indirect one: bleedings tends to be associated with the more difficult cases of footplate removal and the obscured field renders instrumentation less precise and /

and deliberate, the cochlear loss is thus perhaps the result of the instrumental trauma (i.e. a stimulation injury) and only indirectly the result of blood contamination.

The absence of footplate fragments from the cavity of the labyrinth is of direct practical interest; on several occasions fragments undoubtedly fell free into the vestibule and sometimes could even be seen lying deep down with the saccular macula beneath.

It is clear that no harm results from this mishap and there is no justification for hazardous attempts at fishing for fragments. These experiments indicate that lost fragments become adherent to the deep surface of the graft; they must do so whilst it is still not endothelialised, i.e. sometime in the first 10 days, as they are usually incorporated beneath the intact endothelium. They do not stimulate fibrosis nor osteogenesis and their early fixation precludes any possibility of symptoms arising from abnormal stimulation of the vestibular sense organs. Schuknecht (76), in discussing the healing processes involved at the oval window, shares the view that bone chips in the vestibule are entirely inert.

Footplate remains were encountered in five animals, usually at the posterior edge of the window where access in the cat is difficult. Although clearance was thought to have been complete at the time of operation, some of these fragments evidently prolapsed temporarily into the vestibule without losing their ligamentous or periosteal attachments. There was no /

no evidence in this experiment that they stimulate osteogenesis though Schuknecht in the communication just quoted states that such fragments in contact with soft tissues can become a nidus for bone regeneration which may partly close the window. It is desirable to obtain total removal of the footplate but in the present state of knowledge it cannot be said that footplate remains definitely predispose to oval window closure. Nevertheless, Shea has found a significant improvement in his long-term results when complete footplate removal was accomplished (70).

Exposure of the perilymph space to middle ear mucosa which was present in two animals was more likely the result of unsatisfactory positioning of the graft in the inaccessible posterior part, rather than its actual displacement due to perilymph pressure. It was of interest that the mucosa showed no tendency to proliferate in spite of the great facility with which it does so in the middle ear; it had not even spread onto any part of the deep surface of the graft, which had become lined by endothelium in the usual fashion.

One of these animals (Conj.1) showed hair cell damage which was diffuse in character and of moderate severity throughout the organ of Corti. Footplate removal in this animal was considered to have been traumatic and an operative note was made to expect cochlear injury. In addition, electro-coagulation was used on tensor tympani and though there /

there was no other evidence of damage to elements other than the hair cells, electro-coagulation is considered to be the most likely cause: evidence of damage by the mucosal exposure was entirely absent in the other animal and stimulation injury has its own special features which render it easily identifiable. Presbycusis was considered a possibility but was quickly ruled out. According to Schuknecht (89) the epithelial type of this degeneration involves equally and simultaneously all the elements of the organ of Corti, as in man, and spreads from the basal end of the cochlea. It does not simply pick out hair cells at random through the entire length of the organ.

In his extensive studies on the hydrodynamics of the cochlea, Tonndorf (90) has shown by means of cochlear models that maximal displacement of the cochlear partition during manipulations at the oval window occurs in the area corresponding to the lower middle and upper basal turns.

The frequency of damage in this area in experimental animals subjected to stapes manipulations has been reported in the paper of Singleton and Schuknecht (40). In their experiment the stapes procedures were intentionally damaging and included direct needling of the footplate. Cochlear injury was present in four of fourteen animals and was in the site predicted by Tondorf. Damage was most severe in the outer hair cells and less severe in the inner group. The lesions of this type of injury are thus very similar to those of high intensity noise and head injury but they point out that the lesions /



lesions of stimulation injury are less discrete than in true acoustic trauma.

The features of stimulation injury are thus clearly defined and readily recognisable. Though the condition may have been obscured in some of the five animals which sustained severe cochlear damage from electro-coagulation injury or sero-fibrinous labyrinthitis, it was expected to be manifest in certain animals without these conditions in view of the extreme delicacy of the organ of Corti and the close proximity of the basal end of the cochlear duct to the oval window.

As in human stapedectomy, the utmost care was exercised to avoid disturbance but footplate removal was thought to have been traumatic in Sk.2, Conj.3, CT.2 and Ft.1 ("footplate removed after much fishing for fragments, at least one piece was lost completely inside the vestibule"). Yet even in these animals the sense organs were entirely normal on careful histological examination. It is also worthy of note at this point, that no evidence was found to suggest that loss of perilymph was in any way damaging even though loss was severe on several occasions.

The complete absence of stimulation injury in this experiment and the comparative severity of the interference found necessary by Singleton and Schuknecht to produce changes might suggest that the cochlea is relatively resistant to injury in an uncomplicated stapedectomy. However, the incidence of stimulation injury after human stapedectomy is far from insignificant: /



insignificant: in the first series of cases reported by Schuknecht, McGee and Colman (35) limited high tone loss was found in 17.4% of 287 cases though fortunately only in a very small minority was it appreciated subjectively. It would appear that in this instance too close a comparison between animal and human work is to be avoided and that the delicacy of the human organ of Corti must never be under-estimated.

It is evident from this general review and discussion of the changes invoked in the middle ear and internal ear that most changes generally occur independently of the type of graft employed. Several of the conditions observed are caused by the operation itself (i.e. the access and the removal of the stapes) rather than the occurrence of any untoward reaction in these spaces from the actual presence of a graft inserted to replace the footplate.

The only complication which might be specially connected with a particular type of graft is that of sero-fibrinous labyrinthitis occurring with vein. Evidently further work is required to examine the behaviour of this type of graft.

Conditions such as middle ear cyst formation, stapedius adhesion, internal ear blood collections and mechanical and stimulation injury are undoubtedly hazards resulting from the operation rather than the graft's presence. Nevertheless, the only group of three animals in which a satisfactory result was obtained each time were those in which adipose tissue was grafted. This type of graft can be regarded as being eminently /

eminently suitable for use in this situation not only in respect of its own appearance but also because of the lack of any side effects from its presence. These experiments in the cat suggest that it may well be the most suitable of all tissues to use in the human oval window.

## VI. CONCLUSION

In considering the experimental evidence submitted and in drawing conclusions therefrom, it must constantly be remembered that too close a comparison is to be resisted between the short-term results of an operation carried out on the ear of a cat whose oval window and stapes footplate are normal and the long-term results of a similar operation on a human ear, the oval window margins and stapes footplate of which are involved in otosclerosis. Neither must it be presumed that healthy tissues of the cat and the human respond to trauma in a similar fashion. It seems, for instance, that the human cochlea is much more sensitive to injury.

Other factors are also of significance. Firstly, this investigation was limited in scope to a study of three grafts of each type. It is accordingly necessary to avoid being too dogmatic in interpreting the results but now that certain basic facts have been examined on graft behaviour in general it should be possible to design further experiments on a larger scale to examine individual types of graft more closely. Vein certainly needs further evaluation. Additional studies on adipose tissue to examine it in the early post-operative period as well as after many months would also be instructive.

Secondly, the clinical results of human oval window grafting operations are influenced by problems affecting the reconstruction of the sound-conducting mechanism. Though studies /

studies on the tolerance of foreign materials are highly relevant and have shown that the stapes suprastructure can be replaced by a prosthesis, the resultant disturbance of the blood supply to the incus is a problem which has not yet been tackled.

In spite of these limitations, a number of conclusions can safely be drawn from this investigation. It has been demonstrated that although the grafts may undergo modification they remain as definite entities at 18 weeks. The peculiar behaviour of skin renders it unsuitable for use but of the others, adipose tissue consistently gave a good graft, the quality of which was enhanced by the development of its characteristic envelope. The many advantages of adipose tissue do not require further repetition, having been sufficiently listed when evaluating the various types of graft (p. 74 ). Not least among them is the fact that this tissue is easily workable and can readily be incorporated into a prosthesis for human work. The animal experiments suggest that adipose tissue is in fact by far the best tissue for use in oval window grafting. It appears that the remarkable results that can be obtained by stapedectomy and which were first demonstrated with the use of a vein graft, have served to blind otologists to the possibility that stapedectomy with other types of graft (and other methods of reconstruction) might be advantageous. Shea's results with a vein graft are undoubtedly excellent but there are probably few otologists who can repeat them in spite of /

of following his technique very carefully.

It is clear from this study that all the grafts except skin are perfectly acceptable as far as the middle ear is concerned and that all six types of tissue examined are capable of providing adequate oval window closure and securing protection for the internal ear. In spite of the added difficulties of dealing with animals and lack of previous personal experience of this type of micro-surgery, the neuro-epithelial structure of the internal ear remained entirely normal in two-thirds of the animals operated upon. Opening the oval window can thus be a perfectly safe procedure. Failures of one type or another occurred in at least one of the three animals with each type of graft except adipose tissue but it is necessary to differentiate the failures and complications into three groups:

(i) avoidable errors of technique: of these electro-coagulation injury of the internal ear is the most obvious and the most easily corrected;

(ii) failures and complications due to graft failure or an unsatisfactory graft result: these include conditions such as acute labyrinthitis, sero-fibrinous labyrinthitis, excessive fibrosis; certain types of graft probably predispose to these results. A flimsy graft such as mucosa perhaps entails an increased risk of the first; sero-fibrinous labyrinthitis can probably occur with any type of graft, though this experiment suggested that the initiating graft necrosis was more likely with vein; excessive fibrosis may tend to be associated with those grafts which are difficult to position satisfactorily;

(iii) /

(iii) failures and complications due to the operative procedure, i.e. the actual mode of access to the window and removal of the stapes ready for the reception of the graft. These may or may not be of serious import. Excessive fibrosis can arise from difficulty at this point also, if the instrumentation is traumatic in removing the stapes or preparing the graft bed. Conditions such as mucous cyst formation and stapediaal adhesion are clearly unassociated with the actual type of graft. Similarly injury to the internal ear, entry of blood into the internal ear, loss of stapes fragments into the internal ear can occur in other types of operation on this part even though they may not have a complete stapedectomy as their objective.

The significance of these and other mishaps has already been discussed. The pathology of the various failures and mishaps is no less instructive and interesting than the findings in those animals in which a perfectly satisfactory graft-result was obtained. These conditions all have their counterparts in human oval window surgery (with the exception, it is hoped, of electro-coagulation injury) and it is equally important to be aware of the causes and appearances of failure as it is to know the sequence of changes in a successful result. It is reassuring to know that no demonstrable damage is likely from perilymph loss or from blood entering the labyrinth or from footplate fragments falling into the vestibule and that stapes fragments in any situation are probably inert

Thus although the entry of blood or bone fragments into the /

the labyrinth is undesirable it is not dangerous; attempts to retrieve bone fragments are undoubtedly more likely to cause injury than simply leaving them.

From the results of this experiment it is possible to make certain suggestions and comments which are applicable to human work.

(a) Excessive or difficult instrumentation in the oval window area, whether from removing the stapes or positioning the graft, predisposes to fibrosis from mucosal injury. For the same reason excessive reflection of mucosa from the edges of the window is to be avoided.

(b) Minimal scar formation may result in cystic retention of secretions or adhesion of stapedius to the graft. To avoid the latter, it is advisable to divide the stapedius tendon as near to the pyramid as possible.

(c) Any bony fragments in the middle ear are harmless and simply gain attachment to any area of damaged mucosa such as that over the divided stapedius tendon. They do not stimulate formation of excessive fibrous tissue nor become centres of osteogenesis.

(d) During removal of the footplate, the saccule is in danger of rupture. The dangers of direct injury or stimulation injury of the cochlea do not need stressing though these injuries were not encountered in this experiment.

(e) Footplate fragments which fall into the labyrinth do not remain there but become adherent to the graft where, if consisting of normal bone, they appear to remain inert.

"Fishing" /

"Fishing" for lost fragments is thus unjustifiable.

(g) Though blood in the labyrinth is undesirable, it is harmless. It remains in the perilymph space of the cochlea and is removed slowly by phagocytes.

(h) electro-coagulation is to be avoided; even from a distance the cochlea may be in jeopardy.

(i) Perilymph loss during the operations is not of importance. (In fact removal of perilymph has subsequently been found to be advantageous in human operations: its gentle aspiration not only prevents the patient from receiving distressing vestibular stimuli during the footplate removal but also protects the cochlea from stimulation injury caused by those same manipulations or his own involuntary movements.) Perilymph is quickly replaced.

(j) The stapes footplate should be replaced by an adipose tissue graft. It is advantageous if such a graft is slightly small and is then teased out to fill any defects which occur after its natural tendency to spread has been completed. It can be supported or supplemented by gel foam if necessary.

(k) Although the occurrence of post-operative vestibular disturbance frequently indicates cochlear damage, the converse is not true. Severe cochlear injury can occur without vestibular symptoms.

It has indeed now been possible to apply to the treatment of human otosclerosis, information gained in the course of this animal investigation. The results of two series of human operations are included in Appendix II.



REFERENCES

The abbreviations are those given in "World Medical Periodicals" published by the World Medical Association 1957.

- (1) Valsalva, A.M.: Opera, hoc est, tractatus de auro humane, p. 16. Venice (1741).
- (2) Toynbee, J.: Med.-chir. Trans., 24, 190 (1841).
- (3) Toynbee, J.: quoted by Miot in reference 7 (p. 56).
- (4) Kessel, J.: Arch. Ohrenheilk, 11, 199 (1876).
- (5) Kessel, J.: Arch. Ohrenheilk, 12, 237 (1877).
- (6) Boucheron, E.: Bull. med. (Paris), 2, 1225 (1888).
- (7) Miot, C.: Rev. Laryng. (Bordeaux), 10, 49, 83, 113 and 200 (1890).
- (8) Jack, F.L.: Boston med. surg. J., 127, 445 (1892).
- (9) Jack, F.L.: Trans. Amer. Otol. Soc., 5, 284 (1892).
- (10) Blake, C.J.: Boston med. surg. J., 127, 469 (1892).
- (11) Blake, C.J.: Trans. Amer. Otol. Soc., 5, 306 (1892).
- (12) Burnett, C.H.: Med. News (N.Y.), 62, 509 (1893).
- (13) Jack, F.L.: Boston med. surg. J., 128, 8 (1893).
- (14) Dench, E.B.: Trans. Amer. Otol. Soc., 6, 65 (1894).
- (15) Kessel, J.: Arch. Ohrenheilk, 37, 137 (1894).
- (16) Jack, F.L.: Boston med. surg. J., 132, 34 (1895).
- (17) Politzer, A.: Z. Ohrenheilk, 25, 309 (1894).
- (18) Leland, G.A.: Boston med. surg. J., 132, 39 (1895).
- (19) Feraci, G.: Arch. ital. Otol., 7, 129, 225, 313 (1898).
- (20) Feraci, G.: Arch. ital. Otol., 9, 147, 209, 409 (1899).
- (21) Siebenmann, F.: Int. Cong. Med. Paris 1900, 13, 170.
- (22) /

- (22) Sourdille, M.: Bull. N.Y. Acad. Med., 13, 673 (1937).
- (23) Lempert, J.: Arch. Otolaryng. (Chicago), 28, 42 (1938).
- (24) Lempert, J.: Arch. Otolaryng. (Chicago), 43, 199 (1946).
- (25) Lempert, J.: Postgrad. Med., 7, 397 (1950).
- (26) Rosen, S.: Arch. Otolaryng. (Chicago), 56, 610 (1952).
- (27) Davis, H. and Walsh, T.: Laryngoscope (St. Louis), 60, 273 (1950).
- (28) Shambaugh, G.E., Jnr.: Laryngoscope (St. Louis), 62, 461 (1952).
- (29) Lawrence, M.: Arch. Otolaryng. (Chicago), 71, 133 (1960).
- (30) Shambaugh, G.E., Jnr.: Surgery of the Ear, p.498 (W.B. Saunders Co. 1959) Philadelphia and London.
- (31) Rambo, J.H.T.: to be published.
- (32) House, W.F., Glorig, A.: Laryngoscope (St. Louis), 70, 616 (1960).
- (33) Shea, J.J., Jnr.: Laryngoscope (St. Louis), 66, 775 (1956).
- (34) Shea, J.J., Jnr.: Ann. Otol. (St. Louis), 67, 932 (1958).
- (35) Schuknecht, H.F., McGee, T.M. and Colman, B.H.: Ann. Otol. (St. Louis), 69, 597 (1960).
- (36) House, W.F.: Arch. Otolaryng. (Chicago), 71, 156 (1960).
- (37) Portmann, M., Jnr. and Calveric, G.: Laryngoscope (St. Louis), 68, 797 (1958).
- (38) Bellucci, R.J. and Wolff, D.: Ann. Otol. (St. Louis), 67, 400 (1958).
- (39) Bellucci, R.J. and Wolff, D.: Arch. Otolaryng. (Chicago), 71, 224 (1960).
- (40) Singleton, G.T. and Schuknecht, H.F.: Ann. Otol. (St. Louis), 68, 1069 (1959).
- (41) Myers, D., Erulkar, S.D., Schlosser, W.D., Pratt, L. and Winchester, R.A.: Ann. Otol. (St. Louis), 68, 996 (1959).
- (42) /

- (42) Bellucci, R.J. and Wolff, D.: Ann. Otol. (St. Louis), 69, 517 (1960).
- (43) Colman, B.H.: J. Laryng., 74, 858 (1960).
- (44) Withers, B.T.: Personal communication.
- (45) Withers, B.T.: Trans. Amer. Acad. Ophthal. Otolaryng., 64, 863 (1960).
- (46) Hall, I.S.: Personal communication.
- (47) Schuknecht, H.F. and Oleksiuk, S.: Arch. Otolaryng. (Chicago), 71, 287 (1960).
- (48) Wullstein, H.L.: Personal communication; also quoted by Juers, A.L., Laryngoscope (St. Louis), 69, 1180 (1959).
- (49) Zollner, F.: Personal communication
- (50) Zollner, F.: Z. Laryng. Rhinol., 39, 536, (1960).
- (51) Ruedi, L.: Pract. oto-rhino-laryng. (Basel), 22, 11 (1960).
- (52) Walsh, T.E.: Ann. Otol. (St. Louis), 68, 740 (1959).
- (53) Schuknecht, H.F.: Arch. Otolaryng. (Chicago), 58, 377 (1953).
- (54) Kley, W.: Arch. Ohr.-, Nas.-, u.Kehlk. -Heilk., 174, 375 (1959).
- (55) Ruedi, L.: Ann. Otol. (St. Louis), 66, 283 (1957).
- (56) Friedmann, I.: Ann. Otol. (St. Louis), 68, 57 (1959).
- (57) Friedmann, I.: J. Laryng., 69, 588 (1955).
- (58) Escher, F.: Pract. oto-rhino-laryng. (Basel), 16, 32 (1954).
- (59) Hall, I.S.: Personal communication.
- (60) Choremis, C., Economos, D., Papodatos, C. and Gargoulas, A.: Lancet, 2, 437 (1956).
- (61) Wullstein, H.L.: Ann. Otol. (St. Louis), 67, 952 (1958).
- (62) Suggitt, S.: Med. J. Aust., 1, 601 (1955).
- (63) Hall, I.S.: Personal communication.
- (64) /

- (64) Wullstein, H.L.: J. Laryng., 73, 515 (1959).
- (65) Kley, W.: Personal communication.
- (66) Shea, J.J., Jnr.: J. Amer. med. Ass., 174, 2181 (1960).
- (67) Shea, J.J., Jnr.: Personal communication.
- (68) Shambaugh, G.E., Jnr.: Communication to the International Symposium on Otosclerosis, Detroit, 1960 (to be published).
- (69) Schuknecht, H.F.: Personal communication.
- (70) Shea, J.J., Jnr.: Monograph, "Fenestration of the Oval Window after more than Four Years" (1961)
- (71) Shea, J.J., Jnr.: Personal communication.
- (72) Shea, J.J., Jnr.: Communication au 56e Congres Francais d'Oto-Rhino-Laryngologie, Paris, 1958.
- (73) Kos, C.M.: Communication to the International Symposium on Otosclerosis, Detroit 1960 (to be published).
- (74) Kos, C.M.: Ann. Otol. (St. Louis), 69, 559 (1960).
- (75) Hohmann, A.: Communication to the International Symposium on Otosclerosis, Detroit 1960 (to be published).
- (76) Schuknecht, H.F.: Communication to the International Symposium on Otosclerosis, Detroit 1960 (to be published).
- (77) Bellucci, R.J.: Communication to the International Symposium on Otosclerosis, Detroit 1960 (to be published).
- (78) Schuknecht, H.F.: Personal communication.
- (79) Rasmussen, G.L.: J. comp. Neurol., 84, 141 (1946).
- (80) Rasmussen, G.L.: J. comp. Neurol., 99, 61 (1953).
- (81) Portmann, M., Jnr.: Les Fibres Nervouses Efferentes Cochleaires (Imprimerie Librarie Delmos 1951) Bordeaux.
- (82) Schuknecht, H.F. and Weollner, R.C.: J. Laryng., 68, 75 (1955).
- (83) /

- (83) Churchill, J.A. and Schuknecht, H.F.: Laryngoscope (St. Louis), 66, 1 (1956).
- (84) Bellucci, R.J. and Wolff, D.: Laryngoscope (St. Louis), 69, 229 (1959).
- (85) Anson, B.J. and Bast, T.H.: Ann. Otol. (St. Louis), 67, 389 (1958).
- (86) Schuknecht, H.F. and Davidson, R.C.: Arch. Otolaryng. (Chicago), 63, 513 (1956).
- (87) Hohmann, A.: Personal communication.
- (88) House, H.P.: Communication to the International Symposium on Otosclerosis, Detroit 1960 (to be published).
- (89) Schuknecht, H.F.: Laryngoscope (St. Louis), 65, 402 (1955).
- (90) Tonndorf, J.: Laryngoscope (St. Louis), 69, 859 (1959).

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APPENDIX I

The description by Valsalva is of such unusual interest that parts of the passages dealing with the stapes are appended (parts of sections X, XI, XIII).

"The stapes has a special muscle which was discovered by that indefatigable anatomist of the ear, Casserius. Some have said it is not found in man but others have said it is, and they are right. This muscle and that of the malleus are quite obvious amongst the small bones unless they are looked for carelessly or in the wrong place. If anyone attempts to deny without cause the existence of these muscles let him at least observe . . . . .

"The base of the stapes fits into the oval window which it exactly closes; and adheres to it by the intrusion of a thin membrane all round it, but it is not so firm that it cannot be moved up and down through the window as necessary. For I once found in the dead body of a deaf man that the reason for his deafness lay in the fact that this membrane had hardened on to the bony substance, to form one continuous bone with the base of the stapes and the edge of the oval window; and so made it impossible for it to be moved up and down any more . . . . .

"Since I have written that the oval window is closed by the base of the stapes, it must be understood that in the upper part of the tympanum there are two apertures, one of which /



which is the oval window and the other the round window. The oval window has a position parallel to the tympanic membrane and through it there is access to the vestibule of the labyrinth.

"The round window has an indeterminate shape but for the most part tends to be circular. As the first window communicates with the vestibule so does the second with the cochlea; but this is also closed. It is closed however thanks to a thin and small membrane, and this extends not over the actual edge of the window, but over its area a little farther in."

APPENDIX II

Early in the course of the experiment it was decided at the Henry Ford Hospital Detroit, in the Otology Research Laboratory of which the investigation was carried out, that otosclerotic patients would in future be treated by stapedectomy. Initially, the type of graft inserted was connective tissue in nearly every case. However, because of the superior appearance of adipose tissue grafts when the first animal sections became available for preliminary study, it was decided to use adipose tissue in all future human stapedectomies. This policy has been followed ever since.

The author was privileged to take part in this series of human stapedectomies. The results of operation on 287 patients (167 with connective tissue, 106 with adipose tissue grafts) were evaluated by comparing the averages of the post-operative air-conduction threshold for 500, 1000, 2000 c.p.s. and the pre-operative bone-conduction thresholds for the same frequencies. The results were as follows:

Air-bone gap of 5 db. or less:	161 cases (56.1%)
Air-bone gap of 5-10 db.:	54 cases (18.8%)
Air-bone gap of 10-15 db.:	27 cases (9.4%)
	<hr/>
	242 cases (84.3%)

It has also been possible for the author to employ a similar technique in the Royal Infirmary of Edinburgh (in the charge of Dr I. Simson Hall). The results of the first 40 cases /

cases of this smaller series, utilising adipose tissue throughout are:

Complete air-bone closure or better:	22 cases (55.0%)
Air-bone gap of 5 db. or less:	7 cases (17.5%)
Air-bone gap of 5-10 db.:	7 cases (17.5%)

Of the remaining four cases:

1 case (2.5%) is untraced

1 (2.5%) has been made worse than his pre-operative level of 80 db.

1 (2.5%) was known prior to operation to be deaf from extensive adhesions as well as from otosclerosis. Though his hearing improved by 35 db. he still has an air-bone gap amounting to 23 db. for the speech frequency average.

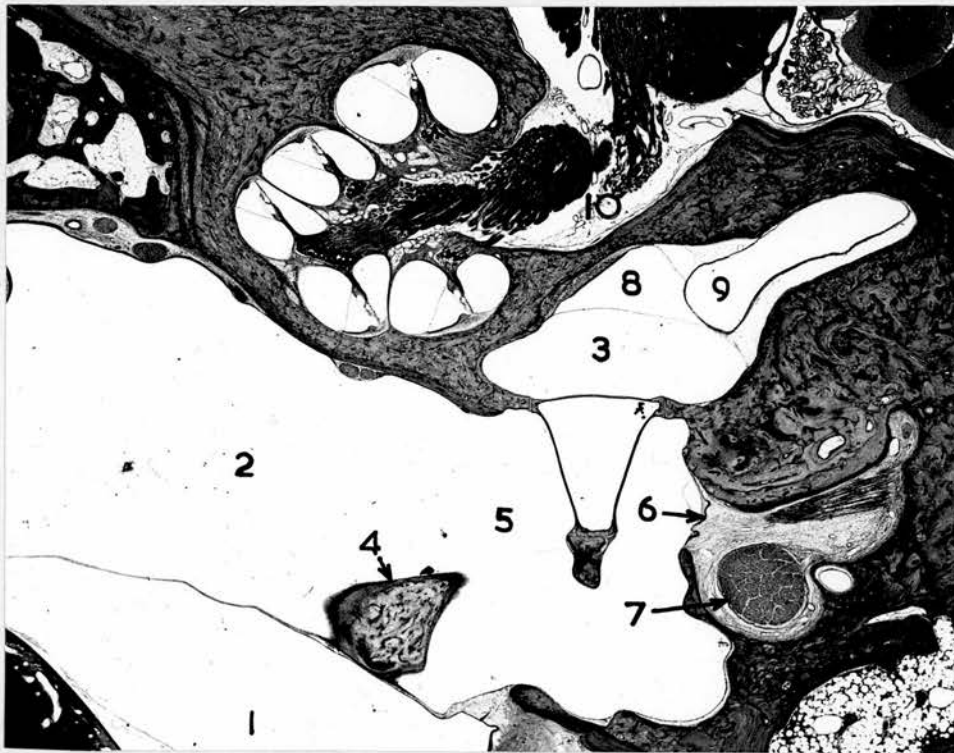
1 (2.5%) improved rather slowly, reached a level within 16 db. of the bone conduction level, then suddenly had a recurrence of severe middle ear deafness 6 weeks after her operation. This case was re-explored very recently but the provisional diagnosis of necrosis of the long process of incus was not confirmed. Instead, it was found that the wire prosthesis had become detached from the incus. It was very simply repositioned and closed more firmly on to the long process. Audiometric re-examination has not yet been possible but the hearing is very obviously much improved again. The re-exploration gave an opportunity for four important observations: (i) the long process of the incus had a very good blood supply, in spite of interference with that part of the supply formerly coming over the stapes. Vessels could clearly be seen coursing down to its tip; (ii) almost the whole of the steel pin was enclosed in a mucosal sheath similar to that described in animals /

animals; the only part not covered was the loop which normally encloses the incus; (iii) there was no evidence to indicate any tendency to bony reclosure of the oval window; (iv) the graft was thin and pliable and still consisted quite obviously of adipose tissue, four months after insertion. Manipulating the steel pin back into position caused slight disturbance of the graft, released fat globules were clearly visible on the mucosa covering the graft. The graft resembled that obtained in animal Ft.3.

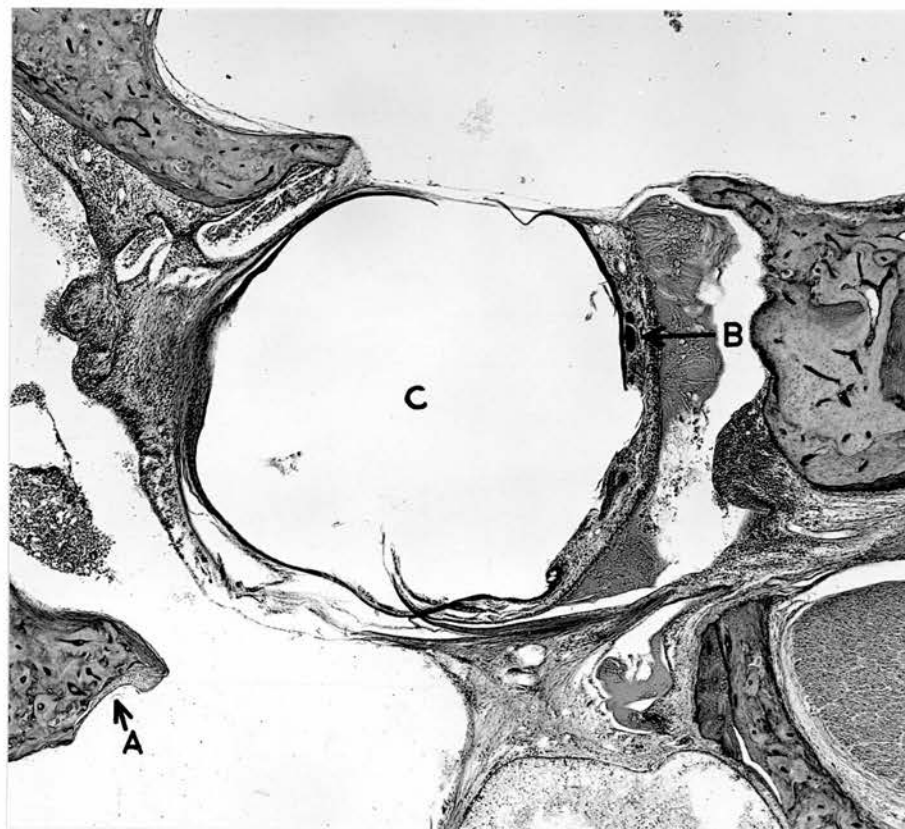
The results are thus encouraging but a long period of observation is clearly necessary before this form of treatment can be adequately evaluated. The results of those cases of the Detroit series in which the first stapedectomy with connective tissue graft was done approximately in May 1959 continue to be maintained virtually unaltered. There is no reason to suppose that those now done in Edinburgh should be any less satisfactory though a period of years will be needed before a final assessment becomes possible.

ILLUSTRATIONS

In all the figures the photograph is orientated so that the top of the photograph is medial and the right edge is posterior (except photographs 35-42). In the case of the high-power views of the grafts, the labyrinthine (endothelial) surface is towards the top edge of the picture and the middle ear surface towards the lower.



**Figure 1.** The normal anatomy of the region seen in transverse section, the cut is below the level of the incus except for the tip of the lentiform process. The section illustrates (1) the external meatus, (2) middle ear, (3) vestibule of the labyrinth. The drum and handle of the malleus (4) are included, also the lentiform process (5) and the stapes. Posteriorly at this point are the stapedius muscle (6) and the facial nerve (7). The saccule (8) includes its macula at this level. The utricle (9) and the beginning of the lateral semicircular canal are visible but the section misses their neuro-epithelial areas. The illustration emphasises the close proximity of the saccule and internal meatus (10) to the stapes footplate. (Control animal A 176, section 50 right. Magnification x 10.)



**Figure 2.** Skin graft.

The graft has formed an epidermoid cyst (C) which occupies the oval window recess. A small amount of dermis is present posteriorly (B). The cyst extends laterally almost to the malleus (A).

(Animal Sk.1, section 130. Magnification x 33).



Figure 3. Skin graft.

The epidermoid cyst (C) has produced an extension (B) into the labyrinth. The section includes the saccular macula, which was normal.

(Animal Sk. 1, section 89. Magnification x 33.)



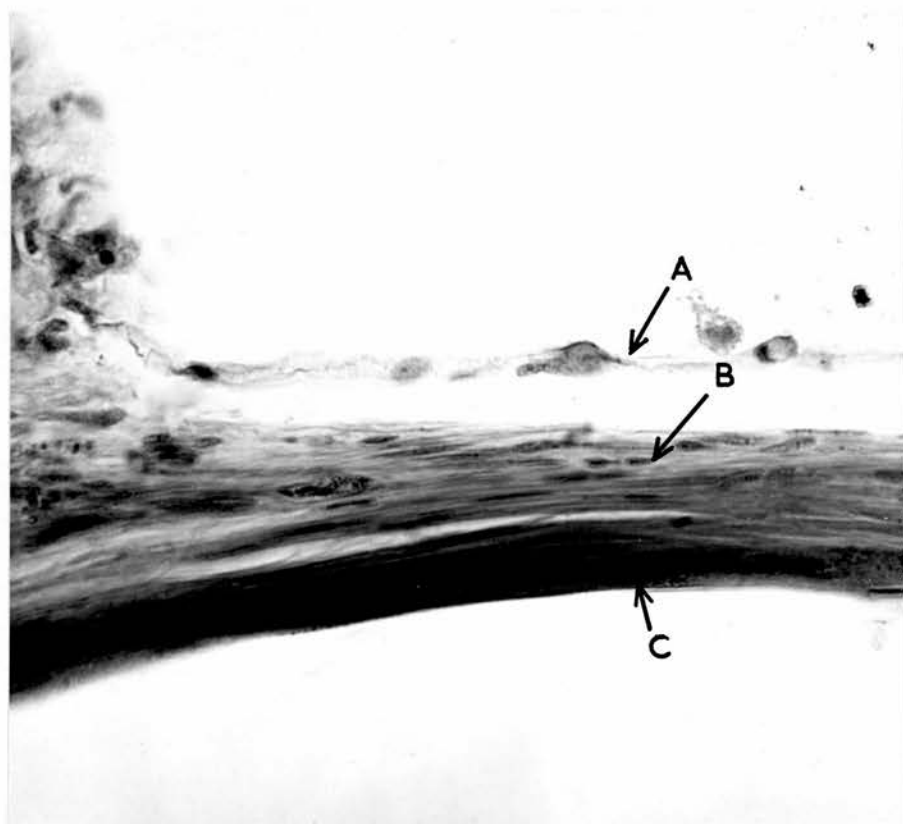


Figure 4. Skin graft.

Section of graft crossing the oval window gap. The labyrinthine surface has an endothelial lining (A). The greater part of the thickness of the graft is provided by the granulosa cell and prickle cell layers (B), which with the corneal layer (C) persist virtually unchanged. (Animal Sk.1, section 130. Magnification x 390.)

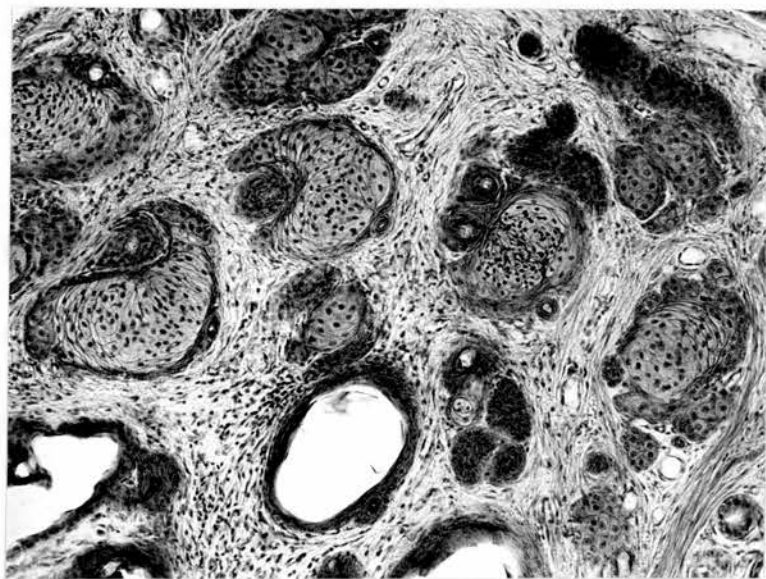


Figure 5. Skin graft.

The section has cut the edge of the cyst at a tangent. At this part the graft has included dermis and contains sebaceous and sudoriferous glands and empty hair follicles.

(Animal Sk.2, section 135. Magnification x 125.)

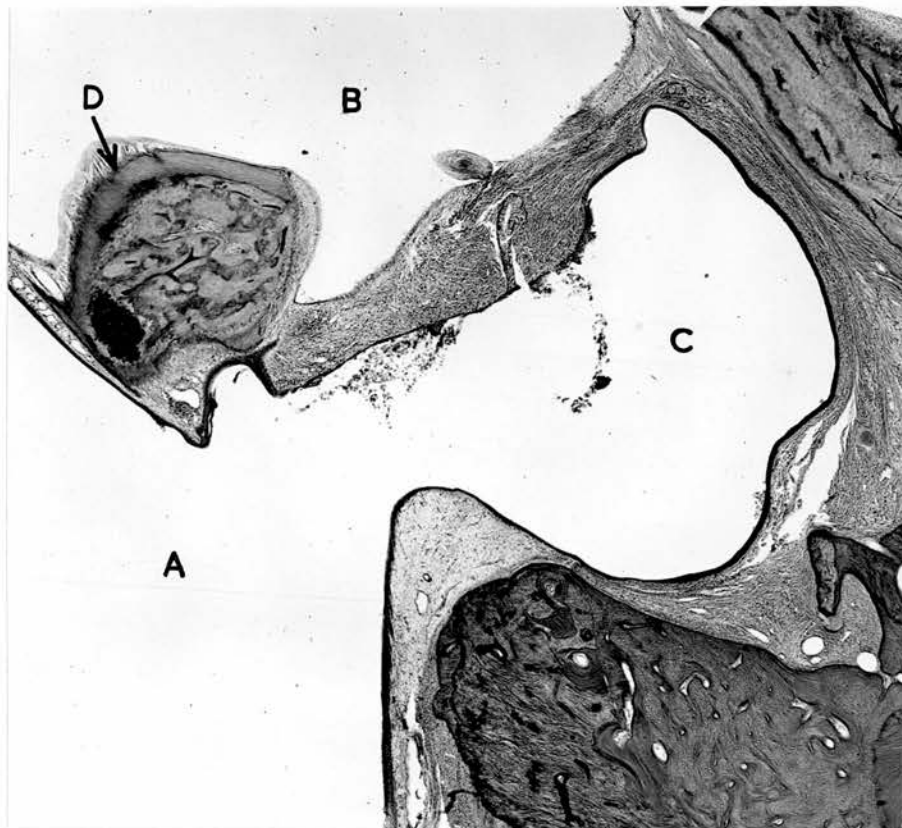


Figure 6. Skin graft.

The epidermoid cyst (C) has perforated into the external auditory meatus (A). As in the other animals in this part of the experiment the middle ear (B) remains undisturbed. The malleus is seen at (D).

(Animal Sk. 3, section 155. Magnification x 33.)



Figure 7. Skin graft.

Collection of epithelial debris contained in  
the epidermoid cyst.

(Animal Sk.3, section 120. Magnification x 390.)



Figure 8. Mucosal graft.

An acute attic suppuration and complete graft necrosis have resulted in an acute labyrinthitis. A saccular tear is present (A) through which polymorphs were swarming.

(Animal Mm.1, section 415. Magnification x 22.)

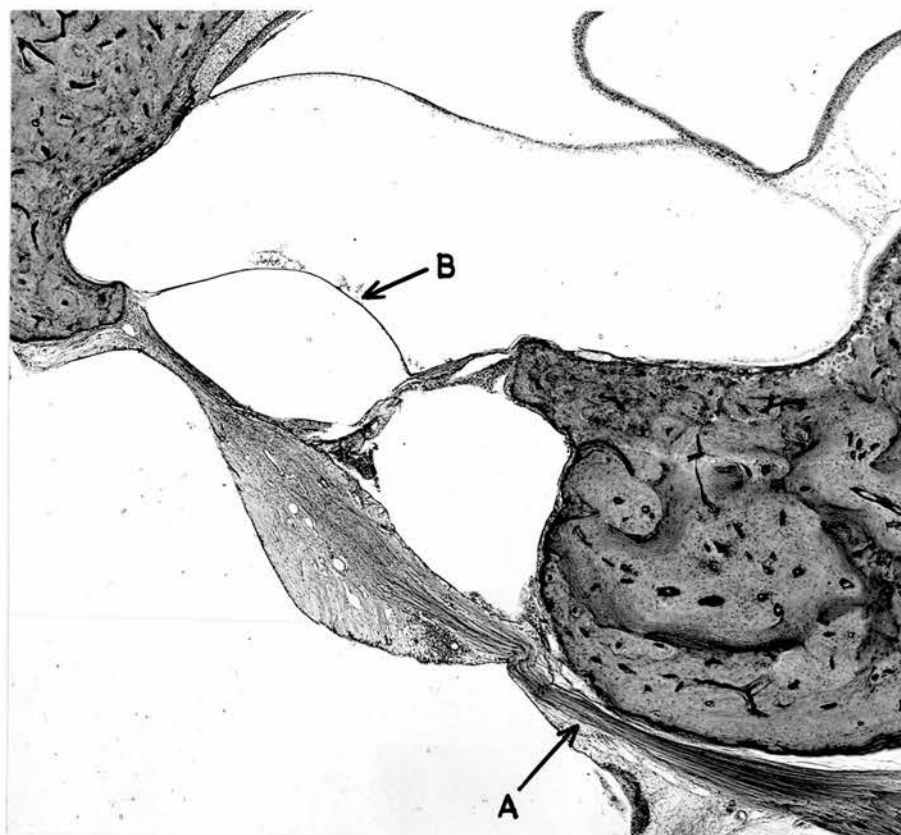


Figure 9. Mucosal graft.

The graft has remained thin but its outline is partly obscured by deposition of collagen which has caused an adhesion to the stapedius tendon (A). The endothelium (B) has become separated from the graft probably during preparation of the sections. (Animal Mm.2, section 95. Magnification x 33.)

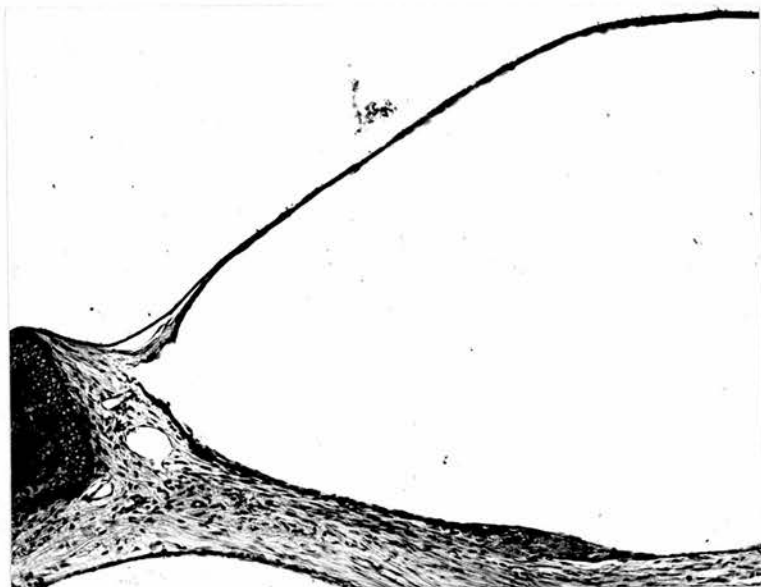


Figure 10. Mucosal graft.

Same animal. Higher power view to show structure of the graft. The endothelial layer (detached) is so thin that its structure is difficult to discern. (Animal Mm.2, section 100. Magnification x 120.)

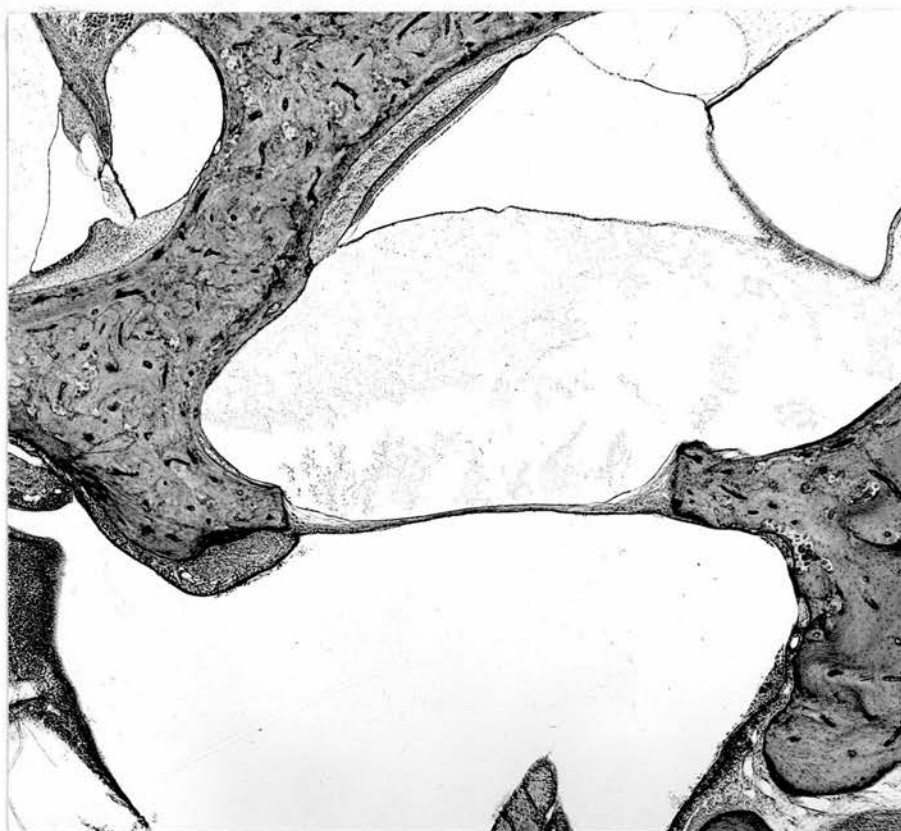


Figure 11. Mucosal graft.

The graft persists as a delicate membrane without having incurred any collagen deposition in this animal.

(Animal Mm.3, section 385. Magnification x 33.)



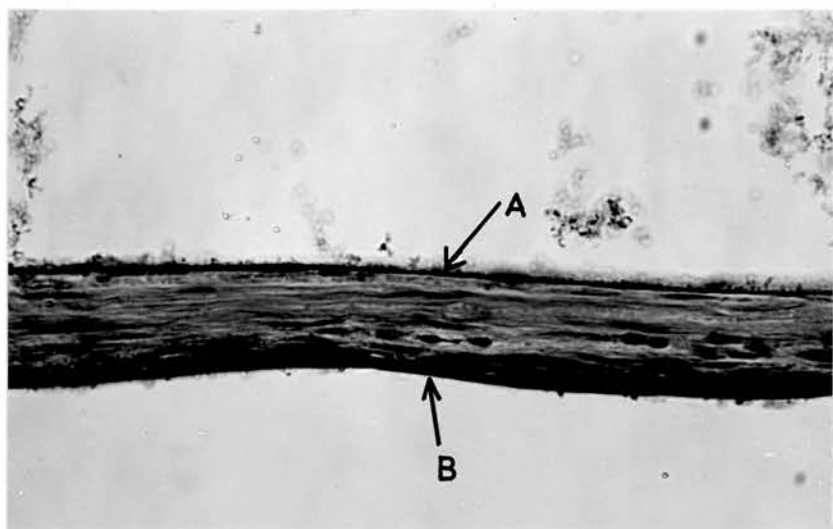
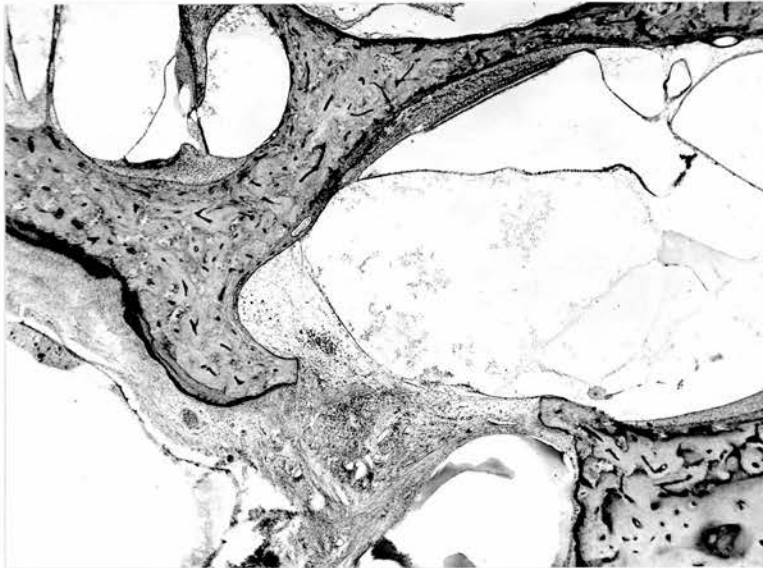


Figure 12. Mucosal graft.

The vestibular surface (A) has a closely attached endothelial lining. The middle ear surface (B) is probably the original epithelial surface of the graft.

(Animal Mm.3, section 385. Magnification x 450.)



**Figure 13.** Vein graft.

An aseptic graft necrosis has occurred with invasion of the vestibule by fibroblastic tissue. (Animal Vn.2, section 110. Magnification x 22.)

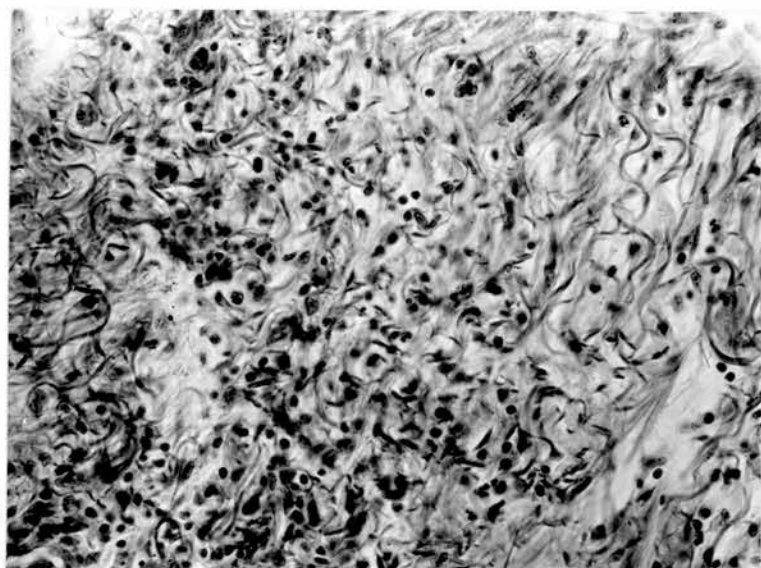


Figure 14. Vein graft.

Section from oval window area of the same animal showing many elastic remnants.

(Animal Vn.2, section 110. Magnification x 300.)

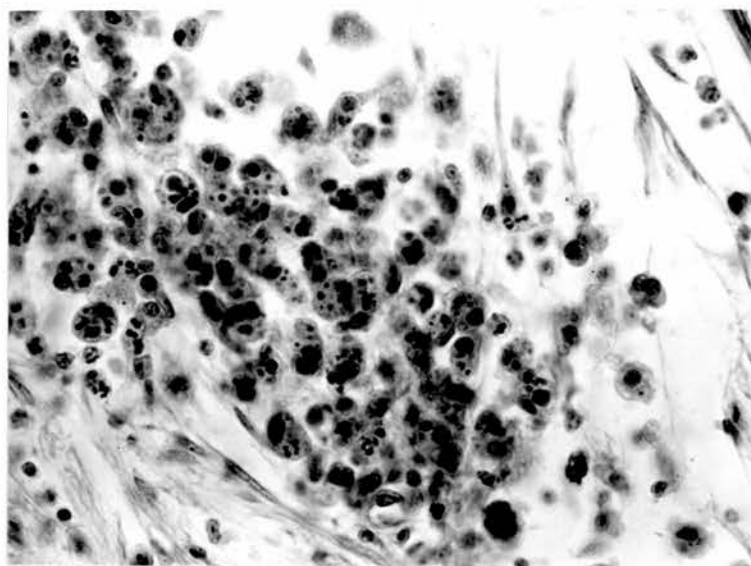


Figure 15. Vein graft.

Section from same animal, just within the anterior lip of the oval window showing typical cell response. The collection consists almost entirely of histiocytes, plasma cells and phagocytes.

(Animal Vn.2, section 110. Magnification x 450.)

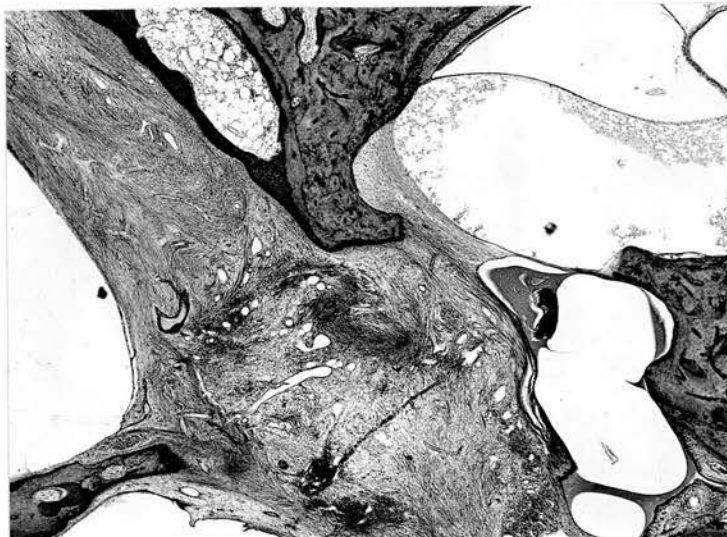


Figure 16. Vein graft.

Extensive fibrosis has occurred anteriorly, extending to the malleus laterally. The posterior part of the window is closed by a thin membrane. (Animal Vn.3, section 90. Magnification x 22.)

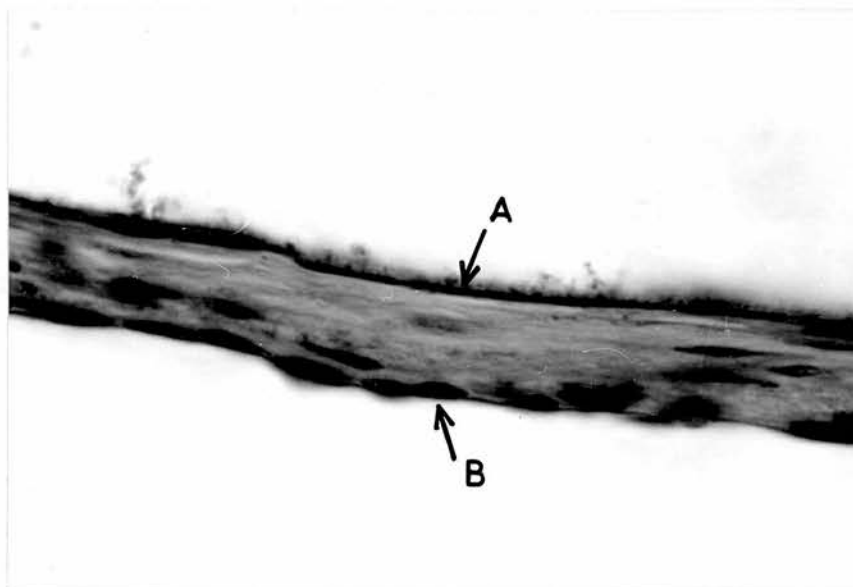


Figure 17. Vein graft.

Section from the same animal. The labyrinthine surface (A) has an endothelial lining. The middle ear surface (B) is probably surviving intima.

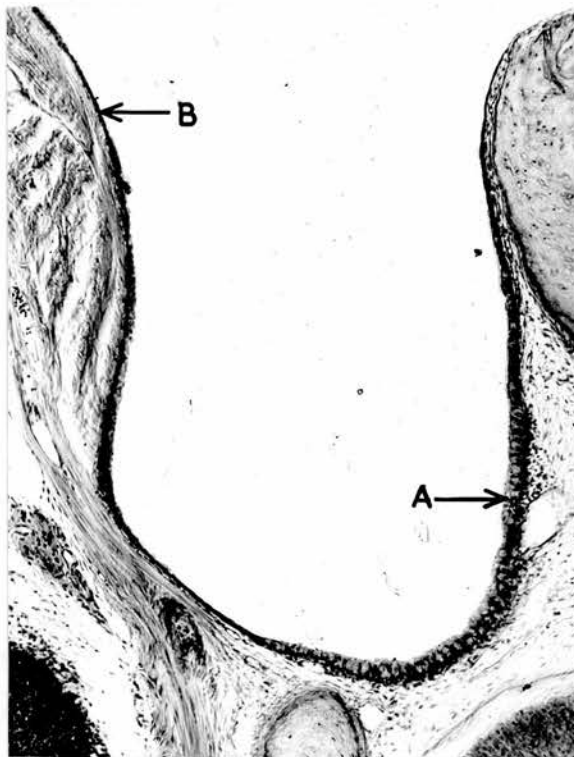
(Animal Vn.3, section 90. Magnification x 1080.)



Figure 18. Conjunctival graft.

The graft (BB) is superficially covered by collagen from which it can easily be differentiated. The vestibular perilymph space (A) has herniated posteriorly (C) as far outwards as the position of the facial nerve (D). Follicular collections of lymphocytes fill the attic.

(Animal Conj.1, section 135. Magnification x 22.)



**Figure 19.** Conjunctival graft.

Same animal. Ciliated columnar epithelium (A) rich in goblet cells is in contact with the perilymph space. The graft itself has an endothelial lining, beginning at (B).

(Animal Conj.1, section 135. Magnification x 85.)



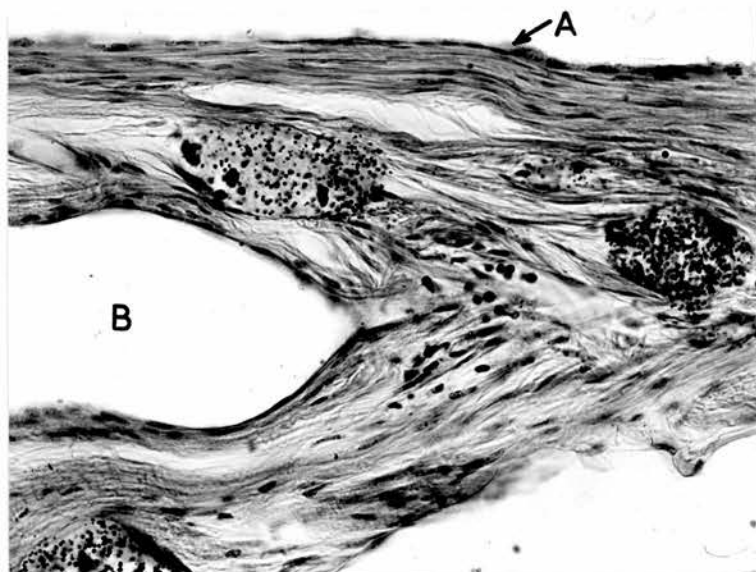
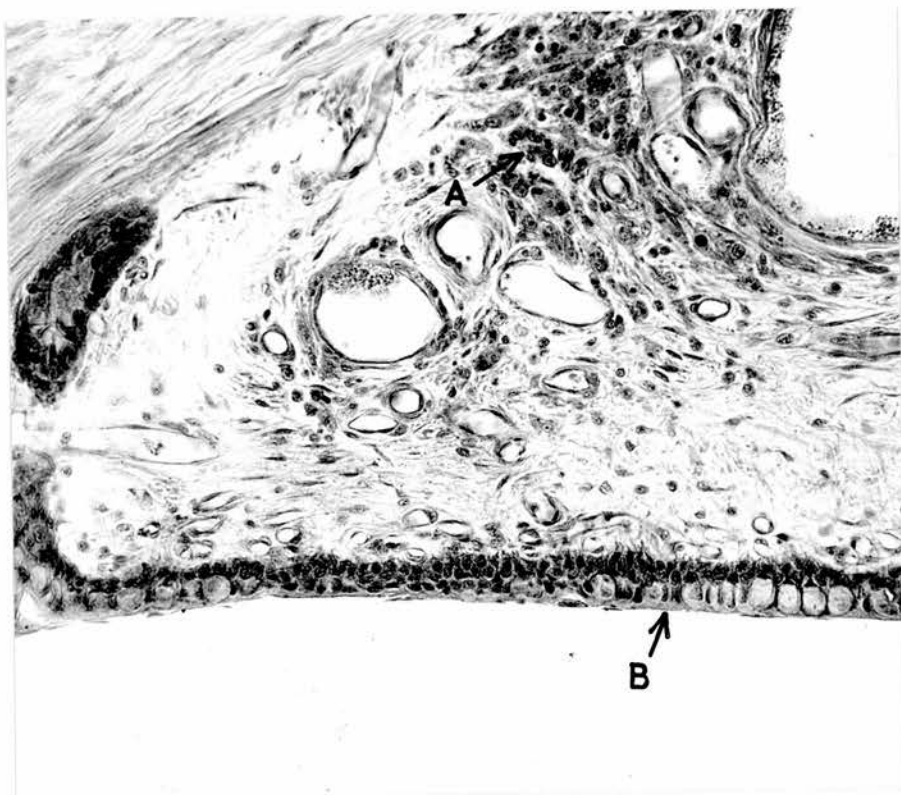


Figure 20. Conjunctival graft.

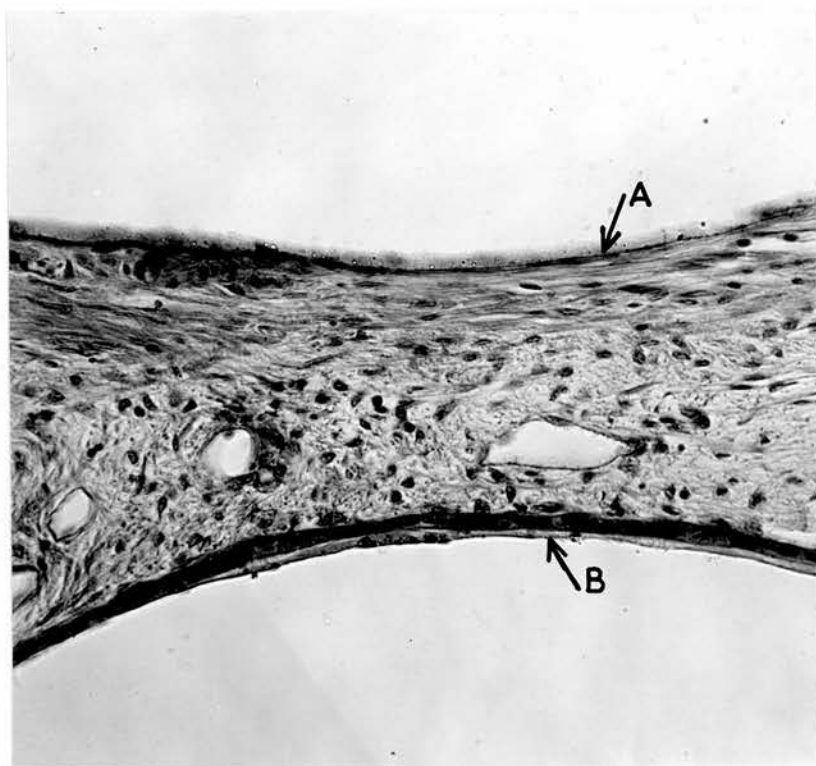
Same animal. The conjunctiva has persisted unchanged. The normality of the endothelium is illustrated at (A). Many vascular spaces were present (B). The aggregations of minute stainless steel foreign bodies have incited no response. (Animal Conj.1, section 135. Magnification x 300.)



**Figure 21.** Conjunctival graft.

Section taken from the very edge of the graft which possessed many melanin-containing cells at this point (A). Vascular spaces again numerous. The edge of the graft has a goblet-containing middle ear type of epithelium (B).

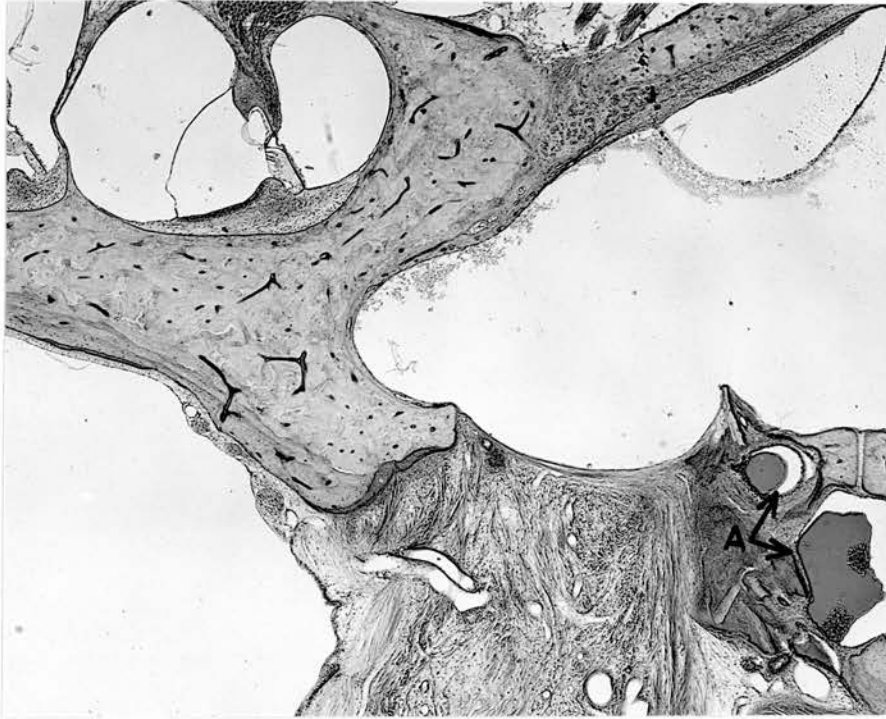
(Animal Conj.2, section 405. Magnification x 390.)



**Figure 22.** Conjunctival graft.

Same animal. Section from central part of graft. Vascular spaces again numerous, endothelial lining (A). The surface presented to the middle ear has a flattened epithelium (B) which is probably the original conjunctival one.

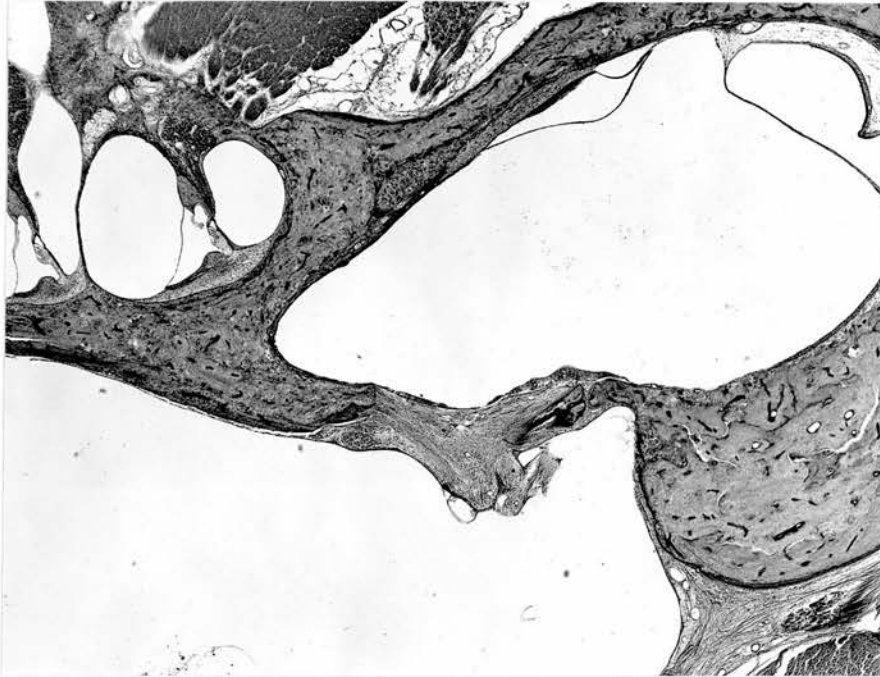
(Animal Conj.2, section 405. Magnification x 390.)



**Figure 23.** Conjunctival graft.

The graft cannot be recognised amid the scar tissue filling the oval window. Posteriorly two mucous cysts are present (A).

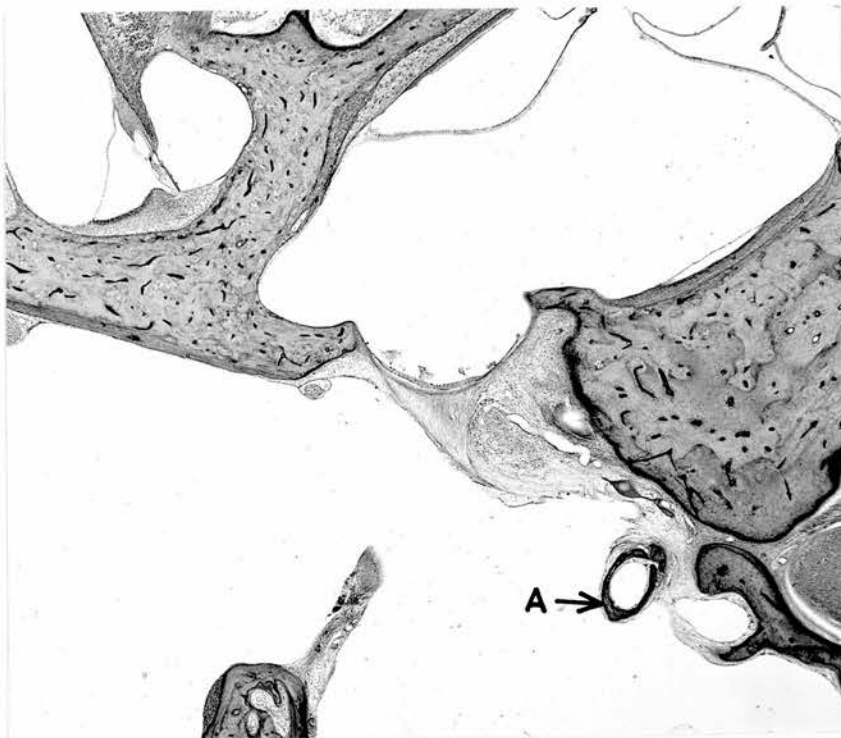
(Animal Conj.3, section 120. Magnification x 33.)



**Figure 24. Connective tissue graft.**

Collagen formation has been just sufficient to  
secure the graft in position.

(Animal CT.1, section 550. Magnification x 22.)



**Figure 25.** Connective tissue graft.

There has been slight excess of collagen formation posteriorly but this is not related to the stapes fragment (A) which has gained attachment to the point of exit of the divided stapedius tendon.

(Animal CT.2, section 130. Magnification x 22.)



**Figure 26.** Connective tissue graft.

Same animal. The endothelium (A) is the site of a granular deposit in the endolymph. Cuboidal middle ear mucosa (B) encloses the superficial surface of the graft.

(Animal CT.2, section 130. Magnification x 390.)

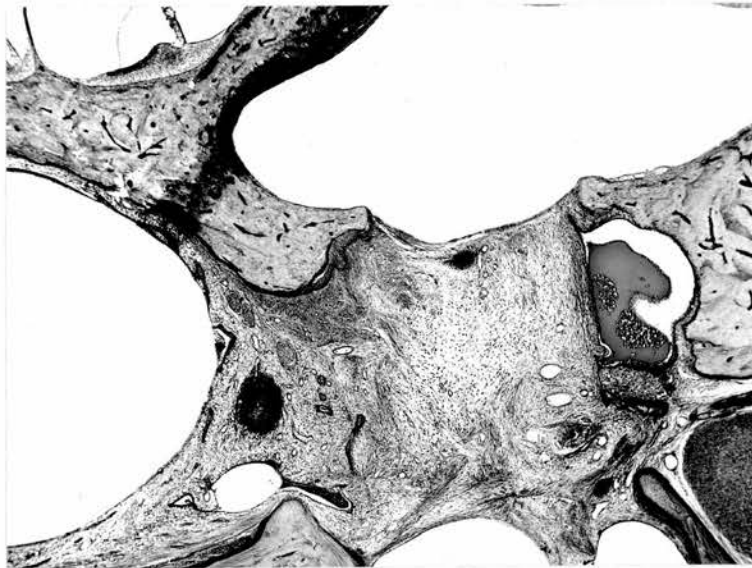


Figure 27. Connective tissue graft.

The entire window recess is filled with scar tissue but this does not encroach into the vestibule. A mucous cyst is present posteriorly.

(Animal CT.3, section 435. Magnification x 22.)



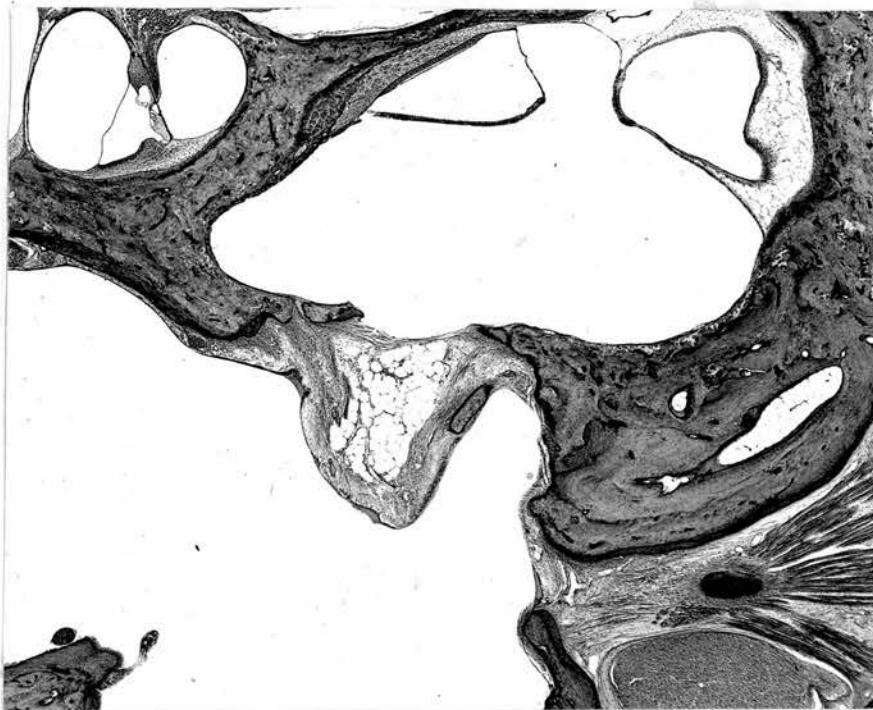
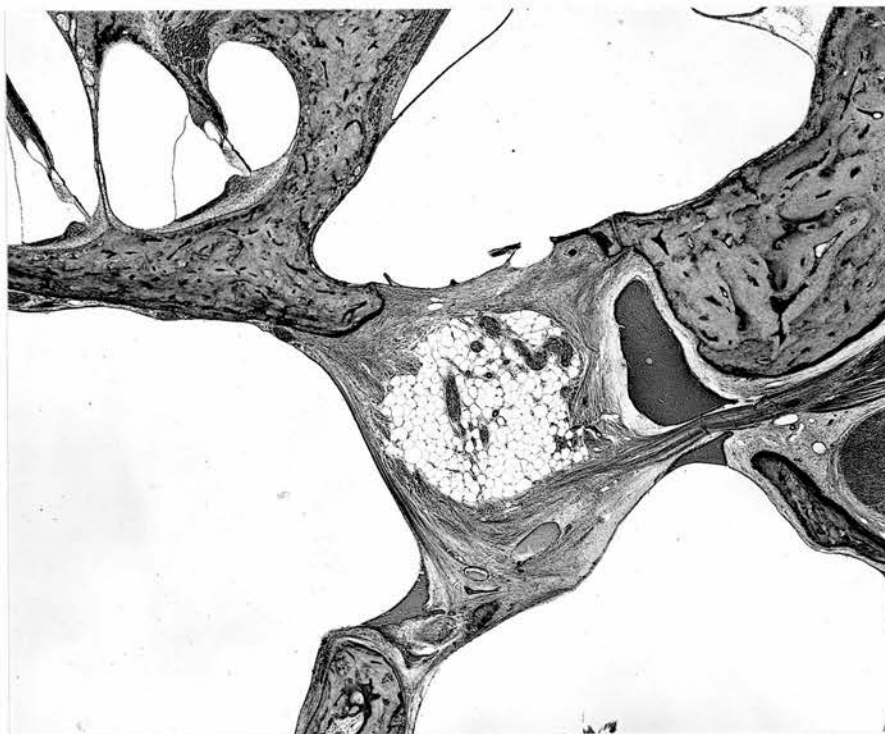


Figure 28. Adipose tissue graft.

The size and shape of the graft in this animal correspond roughly to that of the stapes. The adipose tissue is enclosed in a collagenous envelope, thinnest on the vestibular surface. Footplate remains are present at the window edges. (Animal Ft.1, section 135. Magnification x 22.)



**Figure 29.** Adipose tissue graft.

Similar features as before except that the graft is rather bulkier. Posteriorly there is mucous cyst formation and adhesion to the stapedius.

(Animal Ft.2, section 100. Magnification x 22.)

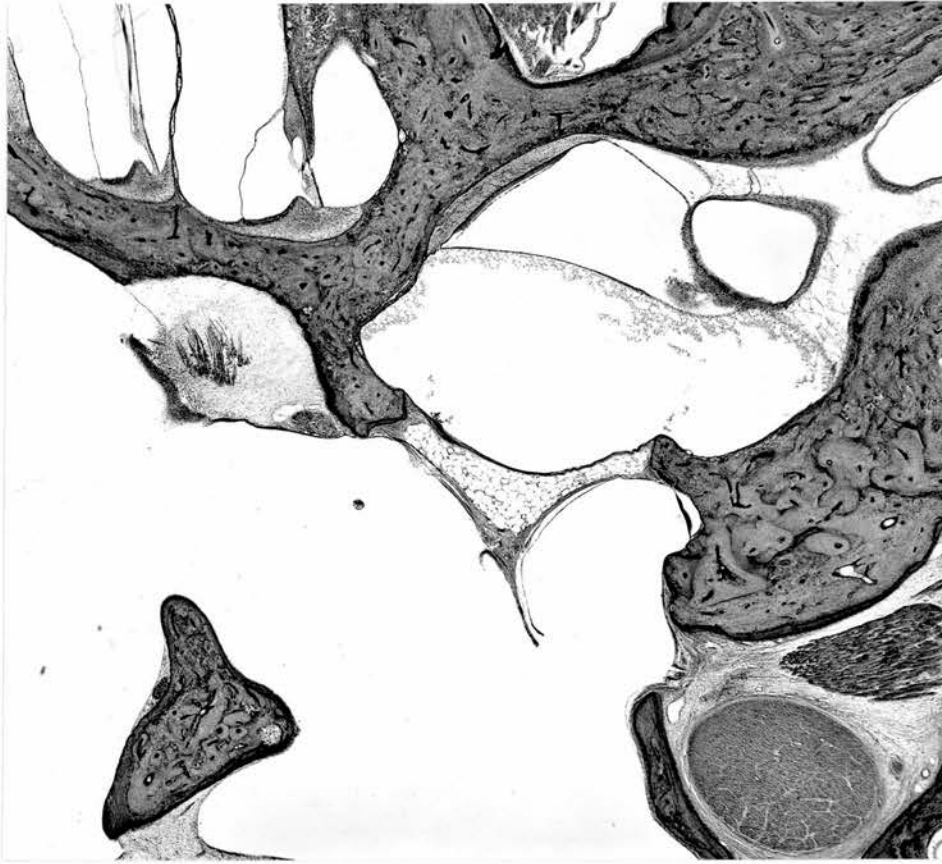


Figure 30. Adipose tissue graft.

The graft was smaller than the previous two and had to be teased out to fill the oval window. The envelope is more delicate than in the others. The soft tissue anterior to the window is part of tensor tympani.

(Animal Ft.3, section 110. Magnification x 22.)

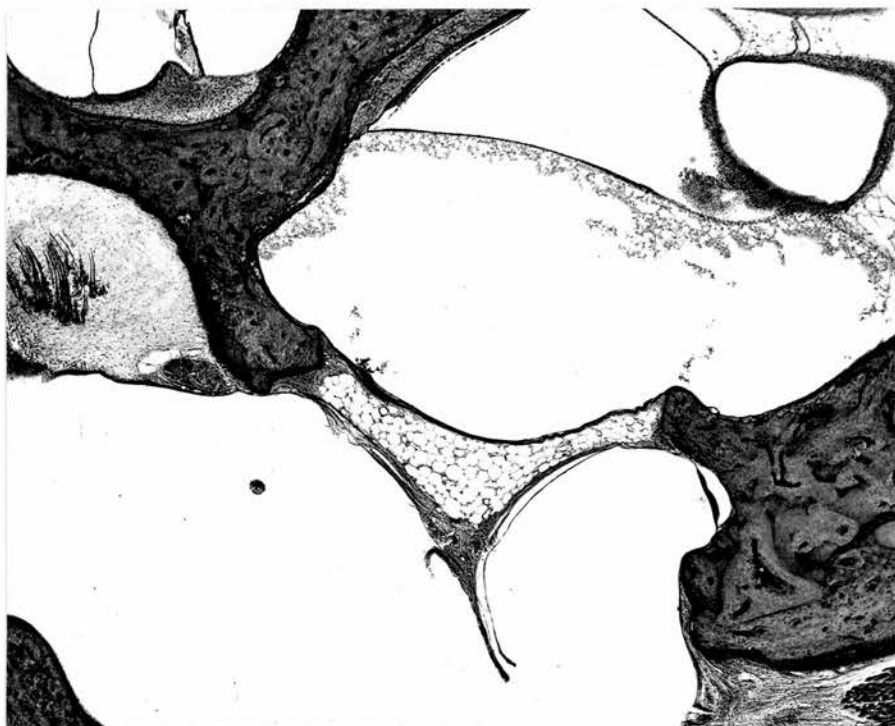


Figure 31. Adipose tissue graft.

Same animal. Oval window area.

(Animal Ft.3, section 110. Magnification x 33)

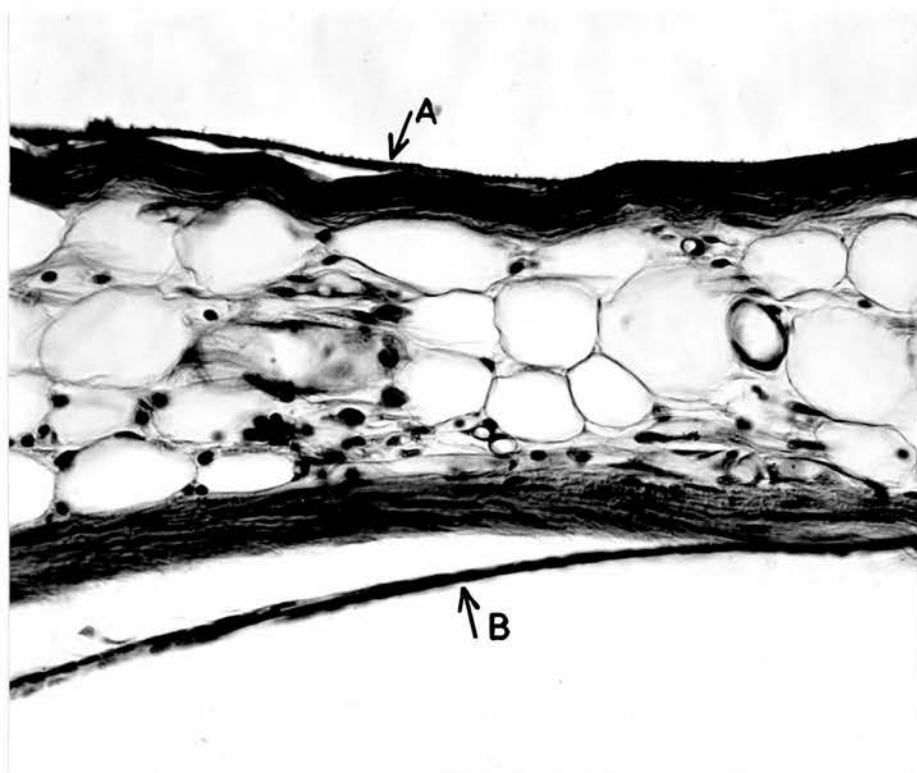


Figure 32. Adipose tissue graft.

Same animal to show details of graft. The envelope consists of mature collagen, no fibroblasts are present in the envelope or between the fat cells, which contain a full complement of fat. Endothelial (A) and epithelial (B) coverings superimposed on the envelope.

(Animal Ft.3, section 110. Magnification x 390.)

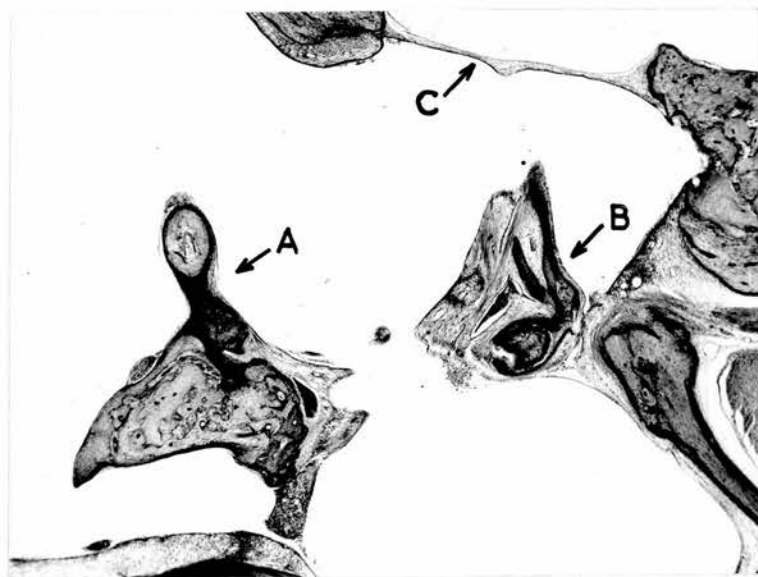


Figure 33. Middle ear: stapes fragments.

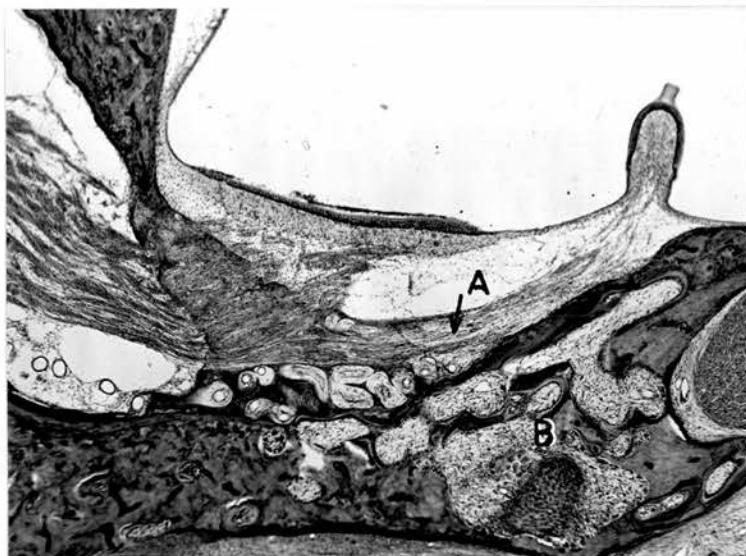
The fragments (B) are enclosed in a small amount of collagen through which they have gained attachment to the area of exit of the divided stapedius. The malleus is represented at (A) and the graft at (C). (Animal Mm.3, section 395. Magnification x 22.)



Figure 34. Middle ear: mucous cyst.

Massive fibrosis has caused isolation of a group of goblet cells at the posterior margin of the window.

(Animal CT.3, section 430. Magnification x 100.)



**Figure 35.** Internal ear: electro-coagulation injury.

In spite of a normal utricular macula and lateral semicircular canal crista and ampullary nerve (A) there are gross changes in the bone of the adjacent labyrinth capsule (B). Small distant foci such as those immediately left of (B) were scattered fairly widely and in adjacent sections could be traced along the vessels.

(Animal Sk.1, section 60. Magnification x 35.)



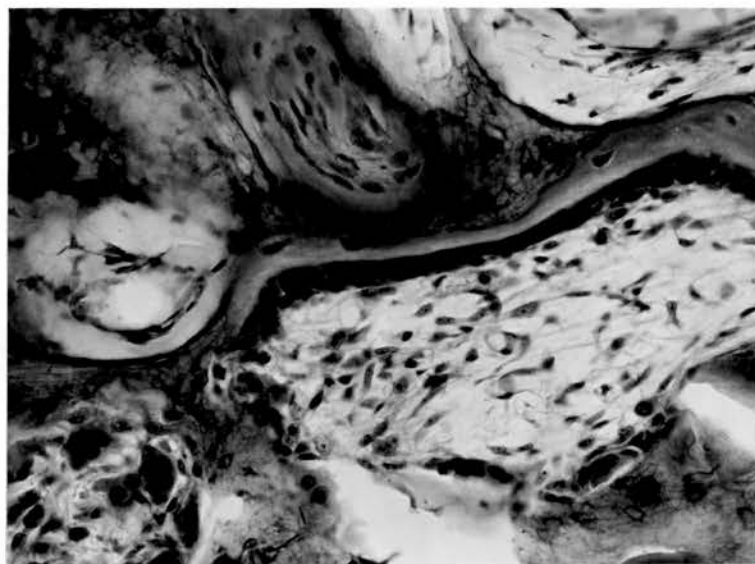


Figure 36. Internal ear: electro-coagulation injury.

Same animal. The widened vascular space contains loose fibroblastic tissue and is lined by many osteoblasts. Osteoclasts are active at lower left. An area of new bone (top centre) is abnormally cellular.

(Animal Sk.1, section 60. Magnification x 300.)

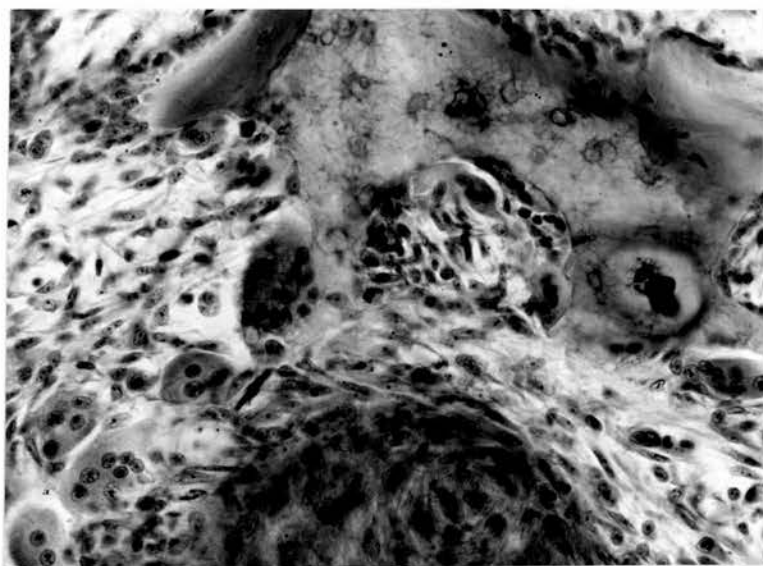
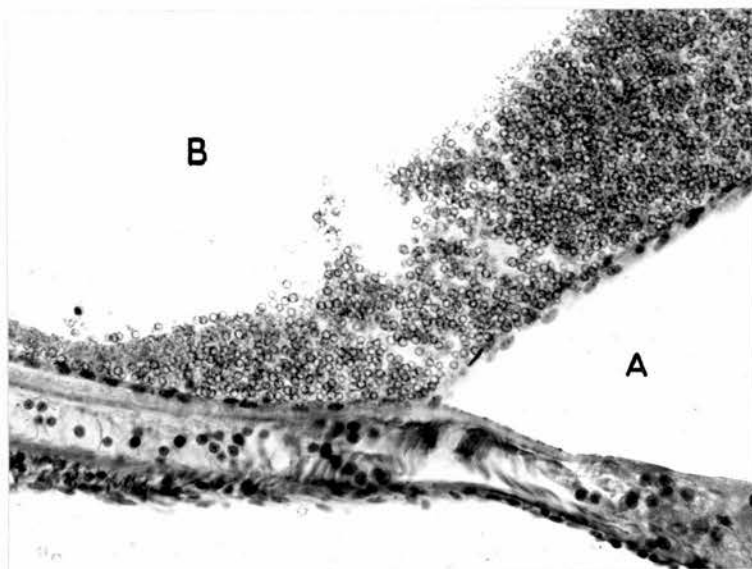
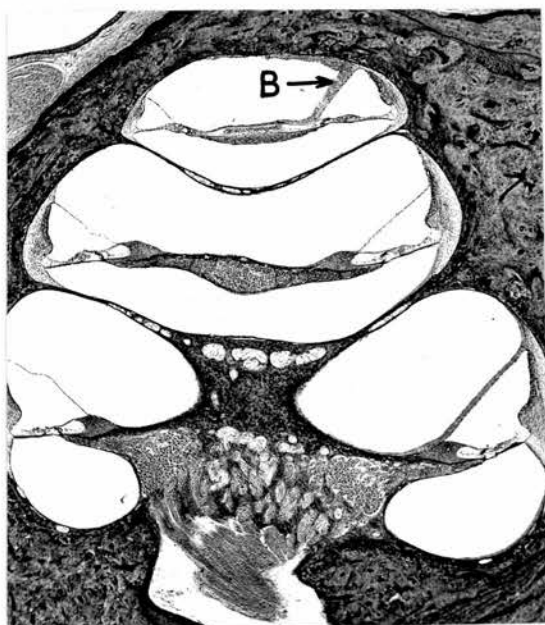


Figure 37. Internal ear: electro-coagulation injury.

Same animal. Another part of the same lesion  
showing mainly osteoclastic activity.

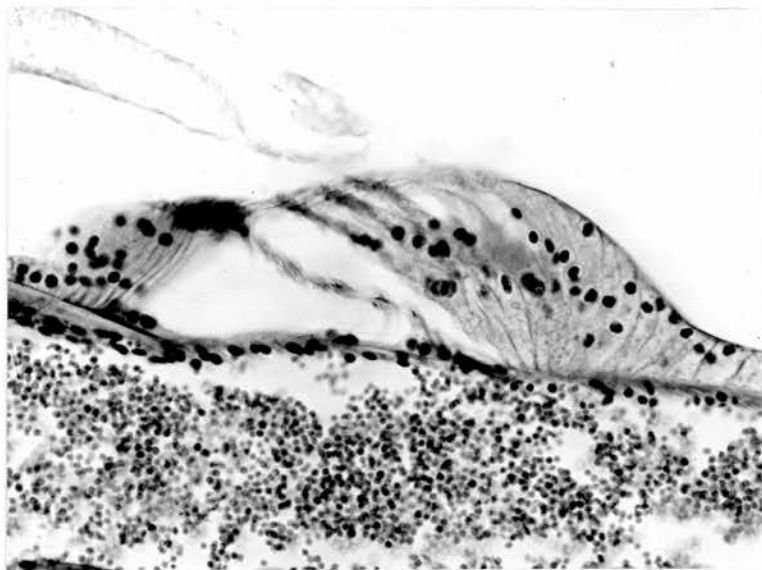
(Animal Sk.1, section 60. Magnification x 300.)



Figures 38 and 39. Internal ear: blood in the cochlea.

The cells were in the scala tympani as well as the scala vestibuli (B) of the apical turn. Cochlear duct (A). The section is rather tangential to the axis of the cochlea.

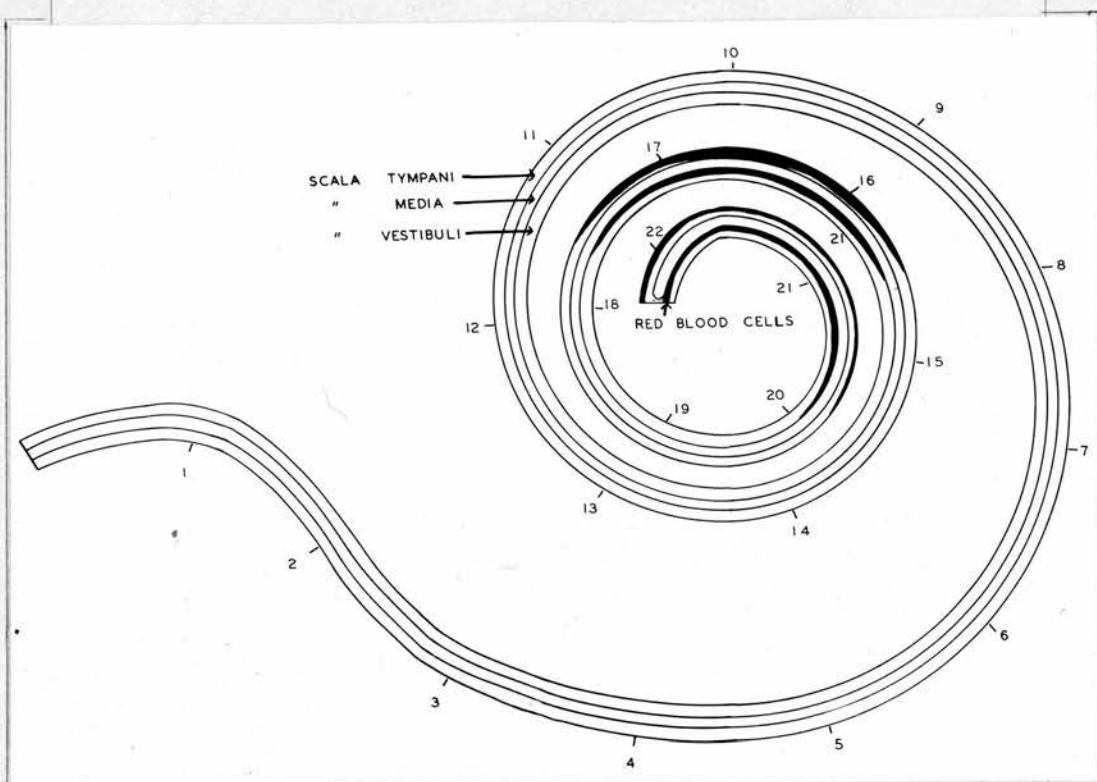
(Animal CT.3, section 402. Magnifications x 22 and x 300.)



Figures 40 and 41. Internal ear: blood in cochlea.

Many cells are visible in the scala tympani of the apical turn (B) and also in the scala vestibuli of the apical turn (A) and middle turn (C).

(Animal Mm.3, section 395. Magnifications x 22 and x 300.)



**Figure 42.** Graphic reconstruction of cochlea.

The distribution and quantity of the blood is represented by the blackened part of each turn: it is in adjacent parts of scalae of the apical and middle turns. The numerals represent the distances in millimetres from the end of the cochlear duct. The duct was of average length in this animal, just over 22 mm.

(Animal Mm.3.)